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IMPACT OF COMPUTER-COUPLED HIGH RESOLUTION MASS SPECTROMETRY ON MOLECULAR STRUCTURE STUDIES[1,2]

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[1] This review represents Part XXIII in the series High Resolution Mass Spectrometry in Molecular Structure Studies. For part XXII, see A. L. Burlingame, P. C. Wszolek, H. K. Schnoes, H. A. Tourtelot, Nature, in press.

[2] Financial support was provided for this research by the U. S. National Aeronautics and Space Administration.

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ABSTRACT

The development of techniques for the acquisition, processing and presentation of entire high resolution mass spectra is reviewed with particular emphasis on the use of real-time digital computers coupled to high resolution mass spectrometers.

Results obtained in this laboratory employing the Nier-Johnson geometry (modified A.E.I.* MS-902) coupled to an S.D.S.** Sigma 7 computer

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* Associated Electrical Industries, Manchester, England

** Scientific Data Systems, Santa Monica, California

demonstrate an accuracy of mass measurement better than 0.5 parts per million for the "4- to 9-average" technique.

Detection and identification of unresolved doublets from error in measurement of the center of gravity is described assuming such accuracy in routine mass measurement.

INTRODUCTION

After wrestling for twenty years in conception, the potentialities held by computers for the alleviation of routine tasks and the logical extension of the human mind are now approaching a stage of infancy,[3]

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[3] For discussion of demanding problems accompanying the application of computers to scholarly disciplines, see Digital Computer Needs in Universities and Colleges, National Academy of Sciences, National Research Council, Publication 1233, Washington, D. C., 1966.

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destined to carry off a revolution in modern society unparalleled in the technological revolution of a century ago. The plummeting drop in cost accompanied by significant increases in performance (high speed, multiplexed input-output, interrupt priority, time sharing, feedback and control, etc.) and reliability have endowed this third generation of digital computers with a nearly ubiquitous market of users in all scientific disciplines. In addition, the versatility inherent in a programmable device over hardware designed for a single task need hardly be mentioned. These features bring the modest investment for a formidable computer system well within the reach of organizations with sufficient resources to afford a research program in high resolution mass spectrometry.

REVIEW

The proliferation of the applications of mass spectrometry pervade the entire scope of organic chemistry, as may be seen in numerous ways. This has most currently been brought into focus by Budzikiewicz, Djerassi and Williams in their volume entitled Mass Spectrometry of Organic Compounds.[4]

[4] H. Budzikiewicz, C. Djerassi and D. H. Williams, Mass Spectrometry of Organic Compounds, Holden-Day, San Francisco, 1967.

This volume makes no claim to review the scope of instrumental and physicochemical techniques now becoming available which will facilitate routine availability of high quality mass spectral data.

Expedience most always dictates a path of least resistance to the actual utilization of information by a wide population of research workers and, hence, iterative solutions are milestones in the history of formidable technical problems, such as this topic of data acquisition, processing and presentation in high resolution mass spectrometry.

In this survey, I wish to review these milestones as I see them, describe the current trends and their successes, and suggest the path of least resistance for the foreseeable future--a forecast strewn with potential pitfalls.

In the ten years since Beynon[5] triggered the application of accurate

[5] J. H. Beynon, in Advances in Mass Spectrometry, Vol. 1, J. D. Waldron, Ed., Pergamon Press, London, 1959, p. 328.

mass measurement to the determination of the atomic composition of organic compounds using a double-focussing instrument, scientists have yearned to bring the full potentialities of high resolution mass spectrometry to their respective disciplines. Such an atmosphere has encouraged the development by commercial manufacturers of instruments with routinely usable resolutions in excess of 30,000--an increase of more than a factor of 3 in the past three years. Current physical dimensions appear capable of about another factor of 2 with usable sensitivity and, of course, higher performance instruments are under development.

While instrument groups were concerned with upgrading spectrometer performance, techniques for data acquisition, processing and presentation became academic problems. The technique of "peak-matching", first used by Nier,[6] allowed accurate measurement of selected, relatively intense

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[6] K. S. Quisenberry, T. T. Scolman and A. O. Nier, Phys. Rev. 102, 1071 (1956).

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peaks under double-focussing conditions. Demand for ascertaining the accurate mass of all peaks in the spectra of complex organic molecules[7a,b]

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[7] (a) P. Bommer, W. McMurray and K. Biemann, 12th Ann. Conf. on Mass Spectrometry and Allied Topics, Montreal, June 7-12, 1964, p. 428; (b) K. Biemann, P. Bommer, D. M. Desiderio and W. J. McMurray, in Advances in Mass Spectrometry, Vol. 3, W. L. Mead, Ed., The Institute of Petroleum, London, 1966, p. 639.

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prompted the development of routine methods for measurement of high resolution mass spectrograms. Automated high precision microphotometers have been developed to such a state *via* data acquisition onto IBM punch cards,[7c] incremental magnetic tape,[8] and on-line to small digital

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[7] (c) A. L. Burlingame, *Ibid.*, p. 701.

[8] R. W. Olsen, 13th Ann. Conf. on Mass Spectrometry and Allied Topics, St. Louis, Mo., May 16-21, 1965.

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computers.[8] While the accuracy of mass measurement has been within usable tolerances (± 3-5 mmu), the photoplate technique suffers from the limitations of being rather time-consuming, while at the same time providing sources of errors due to the fact that the plate must be exposed, developed and measured, before any mass calculations can be accomplished. Another limitation is the historically poor behavior of photographic emulsions in terms of routinely obtaining reproducible relative ion intensity measurements.[9] Techniques for the deconvolution

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[9] R. Venkataraghavan, R. D. Board, R. Kleinowski, J. W. Amy and F. W. McLafferty, in *Advances in Mass Spectrometry*, *Vol. 4*, E. Kendrick, Ed., The Institute of Petroleum, London, 1968, p. 65.

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of partially resolved multiplets in spectrograms, which would improve the virtual resolution of the spectrograph, have been used.[10]

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[10] R. Venkataraghavan, F. W. McLafferty and J. W. Amy, *Anal. Chem.* *39*, 178 (1967); D. D. Tunnicliff and P. A. Wadsworth, *Anal. Chem.*, in press.

To facilitate the mental assessment and comprehension of the vast amount of information contained in a high resolution mass spectrum, several modes of computer presentation have been utilized--all of which first sort the data according to heteroatomic content and then present heteroatomic groups as a two-dimensional array, e.g., "element mapping",[11]

[11] K. Biemann, P. Bommer and D. M. Desiderio, Tet. Letters No. 26, 1725 (1964).

series of plots, e.g., "heteroatomic plotting",[12] or as three dimensional

[12] A. L. Burlingame, EUCHEM Conference on Mass Spectrometry, Sarlât, France, Sept. 7-12, 1965; A. L. Burlingame and D. H. Smith, Tetrahedron, in press.

plots designated "topographical element mapping"[13]--a combination of

[13] R. Venkataraghavan and F. W. McLafferty, Anal. Chem. 39, 278 (1967).

the principles in the first two methods.

Concurrently, the task of acquisition of high resolution mass spectral data from spectrometer instrumentation without use of the feature of a plane of double-focus (spectrograph) has stimulated the development of fast magnetic scanning and concomitant fast response recording systems.

Halbfast and Maurer[14] have cautioned against passing premature judgement

[14] K. E. Halbfast and K. H. Maurer, Abstr. 14th Ann. Conf. on Mass Spectrometry and Allied Topics, May 22-27, 1966, Dallas, Tex., p. 271.

on the relative merits of photographic versus exponential electrical recording of high resolution mass spectral data prior to an understanding of the fundamental differences and trade-offs involved.

Various workers[15] have considered problems associated with fast

[15] A. E. Banner, Abstr. 13th Ann. Conf. on Mass Spectrometry and Allied Topics, May 16-21, 1965, St. Louis, Mo., p. 193; B. N. Green, T. O. Merren and J. G. Murray, Ibid., p. 204.

scanning under high resolution conditions (M/ΔM 10,000; 10% valley). The performance of instrumental resolving power and sensitivity as experienced with fast scanning are generally degraded over their optimum values obtainable under static operating conditions. These problems are inherent in the ion optical aberrations and magnet design, as well as in the amplification and recording systems employed. Theoretical relationships between number of ions per peak and mass measurement precision have been presented.[16] It is shown that for exponential scanning the ion

[16] A. J. Campbell and J. S. Halliday, Ibid., p. 200.

statistics are independent of mass and scan rate (not the case for other scan functions; see later discussion).

Thus far, two appraoches have been investigated which are capable of operating at the high data rates encountered in rapid scanning at actual instrument resolutions in excess of 10,000.

The first approach has involved analogue magnetic tape recording of an exponential magnetic scan of a spectrum using an instrument of Nier-Johnson geometry.[17] Exponential scans from an A.E.I. MS9 of ten and 72 seconds duration per decade in mass have been recorded on analogue magnetic tape from a 10 KHz band pass amplifier--ion multiplier system. This method reportedly yields mass measurement accuracies of about 10 p.p.m., apparently limited by the performance of the tape recording system. Data presented suggests that the low dynamic range of this analogue tape system appears to affect adversely the intensity measurement accuracy. A system employing a FM tape recorder coupled to a PDP-4* computer has been described[18] for digitization of raw data which is subsequently reduced

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[17] (a) C. Merritt, Jr., P. Issenberg, M. L. Bazinet, B. N. Green, T. O. Merren and J. G. Murray, Anal. Chem. 37, 1037 (1965); (b) W. J. McMurray, B. N. Green and S. Lipsky, Anal. Chem. 38, 1194 (1966).

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* Digital Equipment Corporation, Boston, Mass.

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[18] H. G. Boettiger, 15th Ann. Conf. on Mass Spectrometry and Allied Topics, Denver, Colo., May 14-19, 1967.

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by batch processing.

Problems of the nature outlined above have concentrated current effort on the second approach, namely, the development of digital recording systems aimed at sufficient capacity and flexibility to perform the many tasks associated with research in high resolution mass spectrometry.

Initial studies on recording the electron multiplier--amplifier output directly on gapless digital magnetic tape during a magnetic scan indicated the feasibility of the digital technique[19] for high resolution instrumentation.[20] Several groups have discussed systems employing on-line computers utilizing analogue-to-digital converters for direct digitization of multiplier--amplifier output and data acquisition during exponential magnetic scans using A.E.I. MS9 mass spectrometers.[21] Work

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[19] R. W. Olsen and A. L. Burlingame, 13th Ann. Conf. on Mass Spectrometry and Allied Topics, St. Louis, Mo., May 16-21, 1965.

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[20] This technique has recently been shown to be applicable to the recording of conventional mass spectra; see R. A. Hites and K. Biemann, Anal. Chem. 39, 965 (1967); Advances in Mass Spectrometry, Vol. 4, p. 37, Institute of Petroleum, London, 1968.

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[21] (a) W. J. McMurray, S. R. Lipsky and B. N. Green, in Advances in Mass Spectrometry, E. Kendrick, Ed., Vol.4, p. 77; (b) C. Merritt, Jr., P. Issenberg and M. L. Bazinet, Ibid., p. 55; (c) H. C. Bowen, T. Chenerix-Trench, S. D. Drackley, R. C. Faust and R. H. Saunders, J. Sci. Instrum. 44, 343 (1967); (d) H. C. Bowen, D. J. Shields and H. M. Stanier, Advances in Mass Spectrometry, Vol. 4, p. 257.

from this laboratory, [22] employing direct digitization of the multiplier-

[22] A. L. Burlingame, R. W. Olsen and D. H. Smith, 15th Ann. Conf. on Mass Spectrometry and Allied Topics, Denver, Colo., May 14-19, 1967; A. L. Burlingame, R. W. Olsen and D. H. Smith, American Chemical Society - American Physical Society Special Conference on Computers in Chemistry, La Jolla, Calif., June 26-30, 1967; A. L. Burlingame, D. H. Smith and R. W. Olsen, Anal. Chem. 40, 13 (1968), A. L. Burlingame, in Advances in Mass Spectrometry, E. Kendrick Ed., Vol. 4, p. 15.

amplifier output from a C.E.C. 21-110B mass spectrometer and on-line data transfer to a high speed digital computer (both S.D.S. 930 and Sigma 7 computers) has been described in some detail. This system differs from others in that a high precision quadratic magnetic scan was utilized as a scanning function instead of the conventional exponential. This system included a digital, dynamic C.R.T. display of the raw data.

This brings my presentation to focus upon a discussion of current techniques and results from our laboratory.

EXPERIMENTAL

The system currently in use for this work involves the hardware, [Fig 1] or instruments, outlined in Figure 1. The mass spectrometers used are the Consolidated Electrodynamics Corporation 21-110B mass spectrometer and the Associated Electrical Industries MS902 (modified). The C.E.C. instrument is of the Mattauch-Herzog geometry and is used also for our photographic plate work. A magnetic scan function is supplied to the

magnet control circuitry to vary the magnetic field in an approximately known manner. The function presently in use changes the field approximately linearly with respect to time, from high mass to low mass. This means that mass is proportional to time squared. This scan function enjoys two primary advantages over an exponential dependence of mass with time ($m \propto e^T$). The first advantage is that the high mass region, where mass measurement accuracy is most important, is passed through more slowly in a linear as opposed to an exponential magnet current scan. In other words, the interval between two timing marks represents a smaller mass difference. The second advantage is that data reduction is simplified because an equation of $m \propto T^2$ is easy to approximate with a four to five term polynomial. An exponential must, however, be determined in a computer by evaluating a power series until the series converges to the required precision, a slower process. In our particular case, the calculation of accurate masses from real-time data required only minor modifications in our extensive program for photoplate data reduction, since the mass-time dependence is quite similar to the mass-distance function on the photoplate. Figure 2 [Fig 2] presents the general form of the mass vs. time function.

Scanning rates from a few seconds to several minutes for m/e 800 may be chosen.

The output of the electron multiplier during the scan is fed to a field effect transistor amplifier system. The amplified signal is supplied to a high speed analog to digital (A/D) converter that is also capable of multiplexing up to 128 separate inputs (Raytheon Computer Corporation Multiverter). With a cycle time of 40 usec., the maximum digitization rate for a single input (mode for current operation) is 25KHz. The voltage level input (0-10 volts) is converted to a 15 bit (14 bit plus sign) binary number, representing a usable dynamic range of more than 10,000.

A pulse from a variable rate temperature controlled quartz crystal clock determines the initialization of the A/D conversion. This pulse also prepares the computer to accept the converted data. The digitized voltage is transferred to a Scientific Data Systems Sigma 7 computer. The programming involved in handling the input data will be discussed below.

The control system both initializes and terminates the clock count and the scan of the spectrum. Upon termination, the digitized data may be displayed on a C.R.T. display system (Conrac Character Display Monitor, Model CDF) and/or written on digital magnetic tape at the discretion of the operator.

A simplified flow chart of the software, or programming, used in the computer during data acquisition is presented in Figure 3. At the time [Fig 3] the program is loaded into the computer, various parameters are set. The two most important are: (a) a binary threshold is selected at this time for later comparison with a measured voltage level: (b) priority interrupts are set.

Upon receiving an interrupt (external command), the program enters the control phase. This phase processes the interrupt by checking to see that the command is allowed; if allowed, a branch occurs to the particular subroutine requested by the interrupt. These subroutines include those mentioned in the second line of Figure 3, i.e., start scan, stop scan, record and/or display data, and abort.

If the control interrupt requests a start scan function, the program transfers to the data acquisition subroutine. A clock interrupt begins the data cycle. The elapsed number of clock ticks is stored by incrementing a clock register. When the A/D has finished conversion, the digital voltage level is read and compared to the preset threshold. (A more efficient approach under development in our laboratory consists of a circuit external to the central processing unit which determines

whether each data point is greater than threshold, while simultaneously incrementing the clock count, and thereby transfers to the central processor memory only data points which are above threshold.) If it is less than the threshold, the computer waits for the next clock interrupt. If the voltage level is greater than the threshold, the program determines whether or not the previous level was below the threshold. If it was not, the value is simply stored. If it was, both the clock time and the voltage level are stored. The data are stored in one of two buffers. After each datum is stored, the program checks to see whether the buffer is full. If it is, this buffer is written on the magnetic disc of the Sigma 7, while subsequent data are stored in the second buffer. This process results in blocks of data in which peaks are separated by time flags at the beginning of each peak voltage profile.

The process is terminated by an end-of-scan interrupt. At this point, the operator may immediately record the data on magnetic tape or may choose to display the data. The raw digital data may be displayed in a number of ways. The entire spectrum may be presented at once, for example, as a display of peak voltage envelope vs. time. In this mode the spectrum resembles a simple line drawing (or analogue oscilloscopic trace on a storage scope), since the peaks are very narrow compared to the elapsed scan time. Alternatively, the time scale may be expanded to any degree under program control and the peak profiles scanned across the C.R.T. screen sequentially at any chosen rate. This display may be halted at any time by the operator to allow opportunity to study in greater detail peak profiles, dynamic instrument resolution and relative intensities. When the display mode is complete, the spectrum may be either recorded on magnetic tape or aborted. If these data are chosen to be recorded, the blocks of data for the scan are read from the disc and written on digital

magnetic tape. In either case, the computer is automatically reset to idle, at which point the next spectrum scan may be taken.

The digital C.R.T. display, which is located adjacent to the mass spectrometer, has proven to be one of the most important parts of the system in terms of convenience. It provides a "window" whereby the operator can maximize adjustment of instrument parameters to obtain the optimum resolution and sensitivity and the proper beam of calibration compound. It is felt that this type of operator interaction with the system, involving not only the display mode but the degree of control possible in the general scanning procedure represents a unique advantage of a real-time digital data acquisition and processing system. It is also felt that this interaction should be considered in any future system design because of the versatility and enhanced reliability in obtaining optimal quality spectral data, which this permits.

Figures 4a and b present the C.R.T. display in the complete spectrum
[Fig 4a] mode. Figure 4a is the spectrum of perfluorokerosine (PFK), the mass calibration standard, from m/e 500 to m/e 50. From this display the operator immediately knows that sufficient PFK is available for mass
[Fig 4b] calibration. Figure 4b presents the spectrum of PFK and ambelline, an alkaloid of Amaryllidaceae Family, which has a nominal molecular weight of 331. This compound was introduced through the direct insertion lock of the C.E.C. mass spectrometer. It is seen from this display that the spectrum of the alkaloid is of sufficient intensity to permit mass determination of all significant peaks.

Returning to the AEI MS-9 (modified), exponential magnetic scanning was employed utilizing their standard 10KHz bandwidth amplifier in conjunction with a voltage divider circuit which matches the voltage output of the MS-9 to the 0-10 volt range of the A/D.

The basic steps in the data reduction programming are summarized in Figure 5. [Fig 5] As was mentioned previously, this procedure is closely related to that used in photoplate data reduction. The programming is designed to elicit the maximum amount of the available information in a high resolution mass spectrum. The procedure, which is handled entirely by a Control Data Corporation Model 6600 computer system, begins with the raw data tape generated by the Sigma 7 data acquisition system. The tape, with any number of spectra, is read and the data are searched to identify the time flags at the beginning of each peak voltage profile. Peak positions may be determined by two methods currently under investigation: peak tops and centers of gravity.

Peak intensity data may be determined in two different ways, either that given by the maximum peak intensity or the areas, respectively.

The positions and masses of three calibration points at the low mass end of the data are then chosen from the output of the peak position and intensity determination procedure. In the case of quadratic scan spectra (CEC 21-110B), the positions of the rest of the PFK peaks are determined by extrapolation using a three term polynomial of the form $M = A + BT + CT^2$.* When this process is complete, masses of all remaining lines are calculated by interpolation using the calibration points four at a time in a polynomial of the form $M = A + BT + CT^2 + DT^3$. In the case of exponential magnetic scan data (AEI MS-9 modified), the basic equations≠ discussed briefly by McMurray and Lipsky[17b] for

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* It is generally found that this polynomial approximates the mass vs. time dependence accurately enough to predict the time of the next PFK peak within the time width of the peak.

≠ We wish to thank Mr. G. L. Kearns (Picker-Nuclear, White Plains, N. Y.) for providing this information.

the exponential dependence of mass and time were incorporated in our program logic utilized for quadratic real-time and photoplate data.

PFK peaks are then removed from the data and possible elemental compositions for the remaining peaks are determined within error tolerances. This process is accomplished using a computer program outlined here for CHON that may include any of the elements and their isotopes found in organic molecules, such as chlorine, sulfur, etc.

LOGIC FOR RAPID DETERMINATION OF POSSIBLE ELEMENTAL COMPOSITIONS:

1. Specify maximum numbers of atoms wished to be considered: MAX_C, MAX_H, MAX_O, MAX_N

2. Find fractional weights of elements and fractional part of measured mass by subtraction of nominal mass:

   e.g., FW_H = .0078+; FW_O .0051+; FW_N = .0030+, FWT (measured mass)

3. Find limits for possible numbers of H:

$$CMAX_H = \frac{FWT^+ + FW_O \cdot MAX_O}{FW_H}$$

$$CMIN_H = \frac{FWT^- - FW_N \cdot MAX_N}{FW_H}$$

NOTE: MAX_H denotes the maximum specified, while $CMAX_H$ is the calculated maximum.

Where FWT^+ =FWT + measurement error tolerance, e.g., 0.00500 and FWT^- = FWT - measurement error tolerance, e.g., 0.00500.

At this point, certain restrictions can be applied: $CMAX_H$ cannot exceed MAX_H; if it does, set it to MAX_H; if it is negative, there is no composition to be found.

$CMIN_H$ must be at least zero.

Further, considering only CHON, the number of Hs in the composition is even or odd as the sample mass is even or odd* so this correction is applied to $CMIN_H$ and $CMAX_H$ and some of the possibilities in between are also discarded.

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* The program accounts for measured masses that are either slightly below nominal mass or have fractions greater than 0.5.

Finally, if $CMIN_H > CMAX_H$, there is no composition.

At this stage, there are generally only two or three possibilities for the actual number of hydrogens and the program proceeds with heteroatoms O, N.

4. Using the calculated possibilities for number of hydrogens one at a time, the possible numbers of oxygens are calculated:

$$CAMX_O = \frac{(FWT^- - FW_H \cdot {}^{\#}H - FW_N \cdot MAX_N)}{FW_O}$$

$$CMIN_O = \frac{(FWT^+ - FW_H \cdot {}^{\#}H)}{FW_O}$$

Again, the minimum must be at least zero and the maximum cannot exceed MAX_O.

If the maximum is negative or exceeded by the minimum, there is no composition using the present ${}^{\#}Hs$.

5. For each possible combination of the possible numbers of H and O, the possible numbers of N are calculated:

$$CMAX_N = \frac{(FWT^+ + FW_O \cdot {}^{\#}O - FW_H \cdot {}^{\#}H)}{FW_N}$$

$$CMIN_N = \frac{(FWT^- - FW_H \cdot {}^{\#}H + FW_O \cdot {}^{\#}O)}{FW_N}$$

(These two equations are the same, but in actual practice two different values for FWT are used; taking the input tolerances into account.)

As in the case of O, if $MAX_N < 0$ or if $MIN_N > MAX_N$, there is no composition for the present values of H and O.

6. At this point (if there is a possible composition), we have a value for ${}^{\#}H$, ${}^{\#}O$, ${}^{\#}N$.

The program then subtracts:

${}^{\#}H \cdot WT_H + {}^{\#}O \cdot WT_O + {}^{\#}N \cdot WT_N$ from the sample mass and determines whether the remainder is an integral multiple of 12.

If this is the case, a composition has been found and it is printed.* The program then tries another combination of ${}^{\#}H$, ${}^{\#}O$, ${}^{\#}N$; thus finding all possible compositions for the sample given mass.

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* The program rejects all combinations in which ${}^{\#}H > 2 \cdot {}^{\#}C + {}^{\#}N + 3$ or ${}^{\#}C > MAX_C$.

These resulting composition data are sorted according to the heteroatom content of each peak and presented in graphic form using the Cal-Comp Plotter. The details and advantages of this heteroatomic plotting technique, which has been developed specifically to aid in interpretation of high resolution mass spectral data, have been discussed previously.[12]

RESULTS

Mass Measurement. One of the primary investigations of interest was the degree of mass measurement precision and accuracy that could be attained with these systems. This was evaluated at different resolutions, scan and A/D clock rates.

CEC 21-110 Results. Due to instrument sensitivity limitations at the high resolutions used (1:20,000), our studies were concentrated on results obtainable in relatively slow scans that are nevertheless applicable to all situations, including a coupled interrupted-elution gas chromatograph-mass spectrometer system,[23] and direct sample insertion

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[23] R. P. W. Scott, I. A. Fowlis, D. Welti, and T. Wilkins, in Gas Chromatography 1966, A. B. Littleword Ed., Institute of Petroleum, London, 1967, p. 318.

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systems. The particular set of system parameters studied in most detail utilized the spectrum of n-octadecane ($C_{18}H_{38}$). A hydrocarbon was chosen simply because there can be no doubt about the elemental composition and thus the mass of all fragment ions, which makes mass measurement accuracy easy to evaluate. The scan rate was 100 seconds from m/e 300 to m/e 30, at clock rates of 12 and 24 KHz. This yields 30 to 100 data points per peak profile depending on clock rate and mass at a resolution of 1:20,000.

Six successive scans at 12 KHz were recorded and analyzed. The difference in elapsed times between m/e 305 ($C_8F_{11}^+$, m/e 304.98242) and m/e 31 (CF^+, m/e 30.998402) for the six scans was a maximum of 300 clock ticks out of a total of 1.2 to 10^6 ticks. This sort of reproducibility is extremely promising. It indicates that future calibration of a spectrum can be simplified by use of expectation times to identify calibration peaks, rather than the more complex extrapolation procedure used at the present time, which requires that three peaks be specified from the raw data. Analysis of this data, using both peak tops and centers of gravity as peak position criteria, indicates that centers of gravity yield much more precise results. Centers of gravity were used in all calculations which are discussed below. The standard deviations of the determined masses in the six scans averaged about 4 p.p.m., with a worst case of 6.5 p.p.m. and a best case of 2.5 p.p.m. Some representative data are included in Table 1. The data from Table 1 are presented in [Table 1]

[Fig 6] Figure 6. The mean of the six determinations is indicated, and the brackets indicate the standard deviations, both of which are expressed in this case in millimass units rather than parts per million. In general, the greater errors are obtained for peaks of low intensity (less than 5% of base peak, m/e 57). This appears to be a result of the poorer definition of peak profile for these low intensity peaks, resulting probably in part from ion statistical considerations and in part from poorer signal to noise ratio. Mass measurement accuracy is of the order of mass measurement precision as reflected in the means listed in Table 1. It is important to note that errors of this magnitude are sufficiently small to reduce to a considerable degree ambiguities in the determination of elemental compositions.

The smoothness of the mass vs. time function was evaluated in the following manner. Because the calibration points are used four at a time to calculate masses, masses of all peaks except those at the extremities of the calibration range can be calculated by interpolation three times, using three different sets of four calibration points. In no mass region did these three determinations differ significantly from one another, as compared to the standard deviations in Table 1. This indicates that the function is very smooth and is being approximated adequately by the four term polynomial in the present system.

What is most important, however, is that mass measurement errors are only slightly greater than the time resolution of the system in this case. For example, at m/e 30 the time duration between two clock ticks represents a mass difference of about 4 p.p.m. (0.12 mmu). At m/e 300, this mass difference is about 1 p.p.m. (0.3 mmu) (See theoretical error curve, Figure 3.). Results obtained from 24 KHz clock rates (M/ΔM 20,000) indicate that above m/e 70, accuracy a factor of 2 enhanced is routinely attainable.

MODIFIED A.E.I. MS-902 RESULTS[24]

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[24] a) A. L. Burlingame, D. H. Smith and R. W. Olsen, 15th National American Chemical Society Meeting, Division of Physical Chemistry, San Francisco, March 31-April 5, 1968, p. S 269.

b) A. L. Burlingame, D. H. Smith, R. W. Olsen, T. O. Merren, 16th Annual Conference on Mass Spectrometry and Allied Topics, May 12-17, 1968. Pittsburgh, Pa.; Anal. Chem., in press.

This modified A. E. I. MS-902 mass spectrometer is capable of providing a static resolution (M/ΔM) of 1 part in 70,000. Spectra are scanned exponentially by sweeping the magnetic field to cover a selected high mass to low mass range.

In order to assess the accuracy of mass measurements attainable, nine successive spectra were determined at resolution (M/ΔM) 25,000, employing a scan rate of 35 seconds per decade in mass. The multiplier-amplifier voltage output was digitized at 24 KHz. Perchlorobutadiene was chosen for these scans as a compound in which the accurate masses of fragment ions are known unambiguously. The sample flow rate into the ion source from a heated glass inlet system was less than 10 nanograms per second.

For each of these scans, the actual differences between the observed (calculated from PFK interval standard) and the time masses were determined for all peaks in the mass spectrum. The distribution of these actual errors in the resulting 266 calculated accurate mass values covering the mass range 100 to 266 for peaks greater than 2% of the base peak are

[Fig 7] presented in Figure 7 in the form of histograms. Histogram A in Figure 7 illustrates the percentage of errors (absolute values are plotted) falling within the ranges 0-2, 2-4 p.p.m. and so forth. It can be seen that in 70% of the measurements, the observed (calculated) mass differ from the true mass by less than 2 p.p.m.

A significant improvement in precision is obtained by calculating the mean mass for each peak in several scans. If the errors in individual observed masses are random, then both precision and mass measurement accuracy should be improved by a factor of two, by taking the mean mass in four scans, and a factor of three taking the mean mass for 9 scans, etc. If, however, th observed deviations in calculated masses are due to systematic errors in the data acquisition and/or reduction systems, mass measurement accuracy should not be significantly better than for single scans.

In order to statistically evaluate the raw data taken on nine successive scans, the "4-average" mean masses were calculated for scans 1-4 and 5-8. The 58 differences obtained are plotted in Figure 7 as Histogram B. In this case, the error ranges are 0-1, 1-2 p.p.m. and so forth. Comparison with Histogram A shows that the errors are reduced by about a factor of two, with 77% of the errors now less than 1 p.p.m.

The differences obtained from utilizing the mean of all 9 scans are presented as Histogram C in Figure 7, and are plotted over the error ranges 0-0.5, 0.5-1 p.p.m. and so forth. Again the accuracy has been improved. In this case, 60% of the error values are less than 0.5 p.p.m. These results are consistent with the hypothesis that the observed mass measurement accuracy is due to random, rather than systematic errors.

Routine results of real-time data on organic compounds may be illustrated by an evaluation of eight scans of methyl arachidate taken at a resolution of 25,000 over a dynamic range of 500 to 1. For these data, 65% of the mass differences from the mean accurate masses for the eight scans fall within 1 p.p.m. of the true values for those above m/e 100. Considering all masses above m/e 40 in this "8-average" scan, 61% of the mass differences are within 1 p.p.m. of the true values. The largest error noted is that of the isotope peak m/e 44 ($C_2{}^{13}CH_7$), 0.22 m.m.u., or 5.0 p.p.m. The slight decrease in mass measurement accuracy below m/e 100 is relatively unimportant, since there are many fewer possible ambiguities in the assignment of elemental composition.

RELATIVE ABUNDANCE MEASUREMENT:

CEC-21-110B:

Two criteria for relative ion beam abundance have been investigated in some detail, i.e. peak tops and peak areas. Generally speaking, abundances based upon peak tops give good qualitative descriptions of the fragmentation pattern. Current results indicate that peak areas also provide a semiquantitative description of the fragmentation pattern. In addition they provide better reproducibility. Whereas, using n-octadecane as a test compound, six scans gave a standard deviation of relative abundance of approximately 10% of the peak height; analogous data for peak areas under the same instrument conditions gave standard deviation 2-4% of peak height.

It should be pointed out that using a linear magnet current scan in this instrument, the peak time widths increase as a function of $M^{\frac{1}{2}}$. Therefore, the raw measured peak area must be normalized taking this function into account. Of course, such normalization for the exponential

magnet scan are theoretically not necessary assuming constant instrument resolution over the entire mass range.

As far as relative abundance measurements are concerned, the "low resolution" intensities obtained by summing the intensities (peak top) at each nominal mass in the real-time data yield much better agreement with the observed low resolution abundances than do photo-

[Fig 8] plate data. This is illustrated in Figure 8, where the top spectrum (a) is the the low resolution data, (b) the summed real-time abundance and (c) the summed photoplate abundances for the spectrum of 6-hydroxy crinamine- an alkaloid of the Amaryllidaceae family.

MODIFIED A.E.I. MS-902[24]

An evaluation of precision and accuracy of relative abundance measurements for this instrument online to the Sigma 7 was accomplished using the calculated abundance ratios for peaks in the mass spectra of perchlorobutadiene[$(Cl^{35} + Cl^{37})^m$ where $Cl^{35} = 0.758$; $Cl^{37} = 0.242$ and m is the number of chlorine atoms in the species].

Peak abundances may be conveniently expressed as percentages of the total isotopic peaks in a group, i.e.:

$$\frac{n_i}{N} \times 100$$ where n_i is the number of ions in peak i (obtained as discussed in reference 24) and $N = \sum_i n_i$ is the total number of peaks (ions) in the group.

These percentage abundances can be assigned theoretical standard deviations according to the equation:

$$\delta_{theoret.} = \frac{\sqrt{n_i}}{N} \times 100$$

This form of the equation for $\delta_{theoret.}$ results in the standard deviation expressed in the same units (percentage of total ions in the group) as the abundance value itself.

Nine successive scans of perchlorobutadiene at each of the two resolutions (M/ΔM) 11,300 and 30,000, were obtained and these data used to evaluate the accuracy of relative abundance measurements. Representative
[Table 2] data are presented in Table 2 which compare the mean observed abundances with the true abundances, and the observed standard deviations with the theoretical values.

Precision may be evaluated by comparing the observed and theoretical standard deviations in Table 2. It can be seen that they agree very closely, indicating that variations in relative abundance measurements are fully accounted for by ion statistical considerations. This confirms the conclusions reached by McMurray et al.[21a] from a limited number of peaks at 10,000 resolution and extends them to a larger population, wider relative abundance range and resolutions as high as 30,000.

APPLICATIONS TO ORGANIC ANALYSIS

Having at hand a detailed evaluation of a computer coupled high resolution mass spectrometer system (MS-902 - Sigma-7), it is of interest to examine the significance of the "4-average" accuracy of 1-2 p.p.m. in the determination of high resolution mass spectra.

As an example, eight successive scans of the spectrum of methyl arachidate were recorded at 10,000 resolving power (MS-902, 32 seconds per decade with a digitization rate of 24 KHz). Mass measurement accuracy was evaluated in calculating average mass differences (in p.p.m.), between observed and assigned mass, for spectral groups: 1→4, 5→8 and 1→8. Some data representative of those observed for the complete spectrum are
[Table 3] presented in Table 3.

An examination of Table 3 reveals two sets of measurement accuracy data. One set, comprising the majority of the table, includes those data in the 1-2 p.p.m. range expected on the basis of foregoing discussions, the

other set, comprising nominal masses 70, 88, 130, 186, 214, include those data which exhibit very large differences (c.f. errors). These entries may then represent unresolved doublets, with the contribution from a second compositional component shifting the peak center of gravity sufficiently to yield errors larger than expected from the instrument-computer system.

To illustrate this in more detail, the digital peak profiles [plotted from one scan] of the unresolved ^{13}C vs. CH doublet at m/e 42 (ΔM = 0.00446 a.m.u.) and the suspected unresolved doublets at m/e 70, 88 and 130 are pre-
[Fig 9&10] sented in Figures 9 and 10. Peak widths at 10,000 resolution, 35 seconds per decade should be approximately 1500 μsec. The calculated positions of the centers of gravity (labelled C.G., Figures 9, 10) are indicated. The presence of an unresolved ^{13}C isotope peak under the peak profile should result in a shift of the center of gravity to slightly lower mass depending upon the relative abundances of the two components in the unresolved doublet. The profile of m/e 130 in Figure 10 is a particularily striking example of how little the peak profile need be distorted to yield a relatively large mass measurement error, reflected in the center of gravity.

Mass measurement thus provides a criterion for the detection and definition of unresolved doublets. It is important to point out that such large errors may also be observed as the result of not having considered in the algorithm sufficient numbers and kinds of heteroatoms in the computer assignment of elemental composition.

Detection of doublets on this basis depends upon the mass defect of the homogeneous (isobaric) components and the ratio of their abundances. These parameters determine the shift in the observed center of gravity for the unresolved doublet. An equation has been derived to relate this shift, E,

of the larger peak to the abundances I_1 and I_2, and the theoretical component separation, S (reciprical of the theoretical resolution $\Delta M/M$):

$$E = \frac{I_2 S}{I_1 + I_2}$$

This equation is, of course, independent of actual instrument operating resolution.

This equation is used to obtain the error confidence limits in [Fig 11] Figure 11 at the 1 and 2 p.p.m. level. For example, if one is confident that a series of mass measurements should yield an accuracy of better than 1 p.p.m. for single resolved peaks, then doublets with a separation, $S = \Delta M/M$ of 4 p.p.m. are detectable if I_2/I_1, $\geq$ 35%, and doublets with $\Delta M/M$ = 20 p.p.m. are detectable if $I_2/I_1 \geq 5\%$.

This equation may then be applied and tested for the CH *vs.* ^{13}C doublets noted above. The abundance I_2, the ^{13}C contribution, may be calculated on the basis of the corresponding peak one mass unit lower. [Table 4] S and $(I_1 + I_2)$ are known quantities. Data in Table 4 reflect the results of using this procedure to correct the observed error of the doublet for the ^{13}C component in the spectrum of methyl arachidate at an instrument operating resolution of 10,000.

A second application involves the real-time spectra of tetrahydro-N-acetyl pyrrole and three deuterated analogs obtained with the CEC 21-110B. Although current mass spectrometers are not capable of resolving masses differing by two hydrogens *vs.* a deuterium atom at higher masses, the inclusion of an internal mass standard permits mass measurement accuracies capable of distinguishing between the two possibilities. Indeed, it was found that the deuterium content of nearly every peak could be determined by

a simple comparison of differences between observed mass and masses of compositions including any number of deuterium atoms. The lowest difference yields the correct deuterium content. In those few cases where there are significant contributions of both compositions (2 hydrogens *vs.* D) to an observed peak, the observed measured mass generally lies intermediate between the two masses calculated on the basis of elemental composition. Some representative accurate mass data are included in Table 5 and the nitrogen containing fragments and their

[Fig 12] respective deuterium contents are shown in Figure 12. Because these spectra exhibit multiplets at all important peaks below m/e 80 (CH_2 *vs.* N, CH_4 *vs.* O, or NH_2 *vs.* O), interpretation of peak shifts in the spectra of the deuterated compounds is an impossible task on the basis of the low resolution spectra only. Intensities (peak top) of the real-time data, where these multiplets are separated and deuterium content determined, are sufficiently quantitative to yield a clear formulation of the fragmentation processes occurring in such a molecule.[25]

[25] J. M. Tesarek, unpublished results from this laboratory.

A final example is taken from studies of the fragmentation of Veratrum alkaloids,[26] which will serve to illustrate real-time data

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[26] P. Longevialle and A. L. Burlingame, *Bull. Soc. Chim. France*, in preparation.

(CEC 21-110) on natural products of intermediate molecular weight.

[Fig 13] Cevagenine-ring D ortho acetate (Figure 13) has a relatively intense molecular ion at 533 ($C_{29}H_{43}NO_8$). This fragmentation pattern displays a group of intense ions at high mass which are characteristic of the ring-ortho acetate derivatives possible and also a group of relatively low mass fragments containing the E-ring and the heterocyclic nitrogen

CONCLUSIONS

A system for acquiring high resolution mass spectral data in real-time employing a Sigma-7 digital computer has been developed. At this stage of development, real-time data acquisition and display followed by batch data reduction provides extremely accurate mass measurements and relative abundance data. It is felt that this system offers several advantages over previous methods for determining high resolution data, including not only present capabilities (e.g. determination of deuterium, carbon 13, virtual doublets such as C_3 N vs. H_2O_3, etc. solely from mass measurement), but future potentialities for enhancement of accuracy in mass and abundance determinations using the "n-average" technique. The high quality data obtained and presented here, coupled with the flexibility, rapidity and ease of such digital data acquisition, represents a significant state of the art advance in high resolution mass spectrometry.

The use of a digital computer for techniques of this nature offers additional advantages. In a real-time system the capability exists not only for data acquisition but also for a considerable amount of actual data reduction and possible feedback for control while the scan of the spectrum is taking place. An obvious goal is to provide the final output of masses, elemental composition and intensities in suitable form for interpretation in a turn-around time of two or three minutes.

An additional advantage of such a computer is its capability for interaction both with the operator and with the mass spectrometer in terms of control of the various instrument and scanning parameters.

It may be of some interest to note that the data reduction procedure discussed above required, from raw data to final plotted output,

10-12 seconds of C.D.C.* 6600 central processor time for the average spectrum.

It is also apparent that such a data acquisition, processing and interpretative aid is necessary for the implementation of capillary gas chromatographic inlet systems. The interrupted elution chromatographic technique may also prove to optimize the utilization of such large high resolution mass spectrometer-computer systems.

ACKNOWLEDGEMENTS:

I would like to express appreciation to my colleagues without whose ability and conscientiousness this research would not be possible: Dr. Dennis H. Smith, Mr. R. W. Olsen, Mr. R. E. Furey, Mr. John McConnell, Mr. Jan Hauser, Miss Deborah Allen, Mr. Fred Walls and Mr. James Wilder; and to the Lawrence Radiation Laboratory Computer Center for C.D.C. 6600 time.

Also, I wish to thank Mr. T. O. Merren of G.E.C.-A.E.I., Manchester, for the studies involving the modified MS-902.

* Control Data Corp., Minneapolis, Minnesota.

List of Tables

Table 1. Representative Data for Saturated Ions from Six Successive Scans of n-Octadecane

Composition	Exact Mass	Calculated Mass[a]	Standard Deviation[b]
C_3H_7	43.05477	43.05479	4.5
C_4H_9	57.07042	57.07060	3.7
C_5H_{11}	71.08607	71.08634	6.4
C_6H_{13}	85.10172	85.10209	5.2
C_7H_{15}	99.11737	99.11733	5.1
C_8H_{17}	113. 13302	113.13291	6.5
C_9H_{19}	127.14867	127.14810	6.3
$C_{10}H_{21}$	141.16432	141.16450	4.2
$C_{11}H_{23}$	155.17997	155.18139	6.1
$C_{12}H_{25}$	169.19562	169.19741	2.7
$C_{13}H_{27}$	183.21126	183,21172	4.9
$C_{14}H_{29}$	197.22691	197.22717	4.1
$C_{15}H_{31}$	211.24256	211.24224	3.8
$C_{16}H_{33}$	225.25821	225.25876	3.1
$C_{17}H_{35}$	239.27386	239.27348	4.2
$C_{18}H_{38}(M+)$	254.29733	254.29810	5.6

a) mean of six determinations

b) standard deviation of the mean, p.p.m.

TABLE 2

Species	m/e	True Abundance	Resolution 11,300				Resolution 30,000			
			Mean Observed Abundance	Standard Deviation	Theoretical Standard Deviation	Error in Mean Abundance	Mean Observed Abundance	Standard Deviation	Theoretical Standard Deviation	Error in Mean Abundance
C_4Cl_6	258	19.0	18.8	± 0.5	0.5	-0.2	18.8	± 1.3	1.5	-0.2
	260	36.3	36.5	± 0.4	0.7	+0.2	36.6	± 2.2	2.1	+0.3
	262	29.0	28.9	± 0.8	0.7	-0.1	28.7	± 1.6	1.9	-0.3
	264	12.4	12.4	± 0.3	0.4	0.0	12.6	± 0.9	1.2	+0.2
	266	2.96	3.00	± 0.17	0.22	+0.04	3.12	± 0.41	0.61	+0.16
	268	0.38	.35	± 0.12	0.07	-0.03	0.38	± 0.20	0.22	0.00
	270	.02	-	-	-	-	-	-	-	-
C_4Cl_5	223	25.0	25.0	± 0.5	0.4	0.0	24.5	± 1.5	1.2	-0.5
	225	39.9	39.6	± 0.5	0.5	-0.3	40.2	± 1.6	1.5	+0.3
	227	25.6	25.7	± 0.4	0.4	+0.1	25.8	± 0.9	1.2	+0.2
	229	8.16	8.31	± 0.20	0.24	+0.15	8.24	± 0.54	0.68	+0.08
	231	1.32	1.34	± 0.14	0.09	+0.02	1.25	± 0.15	0.26	-0.07
	233	.08	-	-	-	-	-	-	-	-
C_4Cl_4	188	33.0	33.1	± 0.7	0.8	+0.1	32.7	± 1.6	2.3	-0.3
	190	42.2	42.3	± 0.5	0.8	+0.1	42.2	± 2.5	2.6	0.0
	192	20.2	20.3	± 0.8	0.6	+0.1	20.5	± 2.3	1.8	+0.3
	194	4.31	4.35	± 0.27	0.28	+0.04	4.56	± 0.72	0.85	+0.25
	196	0.30	-	-	-	-	-	-	-	-
C_4Cl_3	141	43.6	43.7	± 1.1	1.1	+0.1	43.8	± 5.1	3.3	+0.2
	143	41.7	41.6	± 1.1	1.1	-0.1	42.3	± 2.3	3.3	+0.6
	145	13.3	13.3	± 0.5	0.6	0.0	12.8	± 1.9	1.8	-0.5
	147	1.42	1.38	± 0.28	0.20	-0.04	1.10	± 0.53	0.57	-0.32
C_4Cl_2	118	57.5	56.8	± 1.3	1.3	-0.7	57.7	± 3.6	4.3	+0.2
	120	36.7	37.4	± 1.1	1.1	+0.7	36.0	±3.9	3.4	-0.7
	122	5.80	5.81	± 0.48	0.42	+0.01	6.30	± 1.37	1.41	+0.50

TABLE 3

Mass Measurement Accuracy, Methyl Arachidate

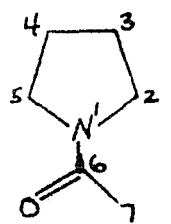
Assigned Composition	Assigned Mass	Average Difference(in p.p.m.) 1-4	5-8	1-8	Average Intensity
C_3H_5	41.03912	- 0.51	- 1.62	- 1.06	7.81
$C_2{}^{13}C_1H_4$	42.04247	- 0.48	- 1.97	- 1.32	0.26
C_3H_6	42.04694	- 1.03	0.26	- 0.39	2.49
$C_2H_3O_2$	59.01330	1.32	0.85	1.08	1.43
C_5H_9	69.07042	- 0.53	- 0.37	- 0.45	12.45
C_5H_{10}	70.07824	-11.86	-12.53	-12.20	3.50
$C_3H_6O_2$	74.03677	0.27	- 0.95	- 0.34	100.00
$C_4H_7O_2$	87.04459	0.43	0.68	0.50	65.14
$C_4H_8O_2$	88.05242	-27.48	-25.30	-26.39	3.91
$C_5H_9O_2$	101.06024	- 0.35	0.67	0.16	0.81
C_9H_{13}	121.10172	- 0.76	- 1.55	- 1.15	0.98
$C_7H_{13}O_2$	129.09155	- 1.52	- 0.38	- 0.95	7.19
$C_7H_{14}O_2$	130.09936	- 9.03	- 7.87	- 8.45	2.10
$C_8H_{15}O_2$	143.10719	0.25	- 0.37	- 0.06	19.84
$C_{12}H_{19}$	163.14866	- 0.18	0.82	0.32	0.39
$C_{11}H_{22}O_2$	185.15414	0.28	- 0.09	0.10	4.93
$C_{11}H_{23}O_2$	186.16196	-12.79	-12.21	-12.45	1.13
$C_{13}H_{25}O_2$	213.18544	0.62	- 0.13	0.25	2.05
$C_{13}H_{26}O_2$	214.19326	-10.05	- 8.68	- 9.38	0.70
$C_{18}H_{34}$	250.26603	- 0.48	1.17	0.25	0.18
$C_{18}H_{35}O_2$	283.26368	0.25	- 0.46	- 0.10	8.82
$C_{19}H_{37}O_2$	297.27933	- 0.51	0.47	- 0.02	1.82
$C_{21}H_{42}O_2$	326.31846	- 0.28	- 0.63	- 0.45	15.34

Table 4

Nominal Mass	Observed Error (p.p.m.)	Mass Measurement Error, CH Species (p.p.m.)
70	-12.20	0.2
88	-26.39	-0.1
130	- 8.45	-0.3
186	-12.45	-0.3
214	- 9.38	-0.8

Table 5 Representative Data from Unlabeled and Labeled N-Acetyl-tetrahydro-pyrroles

Ion[a]	Calculated Mass[b]	Possible Composition[c]	Exact Mass	Diff.[d]
M+(d_o)	113.08380	$C_6H_{11}NO$ *	113.08406	0.2
		$C_6H_9NO\ D_1$	113.082421	-1.4
M+(d_{2a})	115.09600	$C_6H_7NO\ D_3$	115.09480	-1.2
		$C_6H_9NO\ D_2$ *	115.09643	0.4
		$C_6H_{11}NO\ D_1$	115.09807	2.0
M+(d_{2b})	115.09682	$C_6H_7NO\ D_3$	115.09480	-2.0
		$C_6H_9NO\ D_2$ *	115.09643	-0.4
		$C_6H_{11}NO\ D_1$	115.09807	1.2
M+(d_3)	116.10333	$C_6H_8NO\ D_3$ *	116.10262	-0.7
		$C_6H_{10}NO\ D_2$	116.10426	0.9
m/e 98(d_o)	98.06106	$C_5H_6NO\ D_1$	98.05897	-2.1
		C_5H_8NO *	98.06058	-0.5
m/e 100(d_{2a})	100.07263	$C_5H_6NO\ D_2$ *	100.07296	0.3
		$C_5H_8NO\ D_1$	100.07460	2.0
m/e 100(d_{2b})	100.07280	$C_5H_6NO\ D_2$ *	100.07296	0.1
		$C_5H_8NO\ D_1$	100.07460	1.8
m/e 98(d_3)	98.06121	C_5H_8NO *	98.06058	-0.6
		$C_5H_6NO\ D_1$	98.05895	-2.2

a) d_o indicates unlabeled molecule, 

; d_{2a} indicates 2,2-d_2;

d_{2b} indicates 3,3-d_2;

d_3 indicates 7,7,7-d_3

Table 5 (cont'd)

b) from real-time data

c) * indicates assigned composition

d) Diff. in millimass units (m.m.u.)

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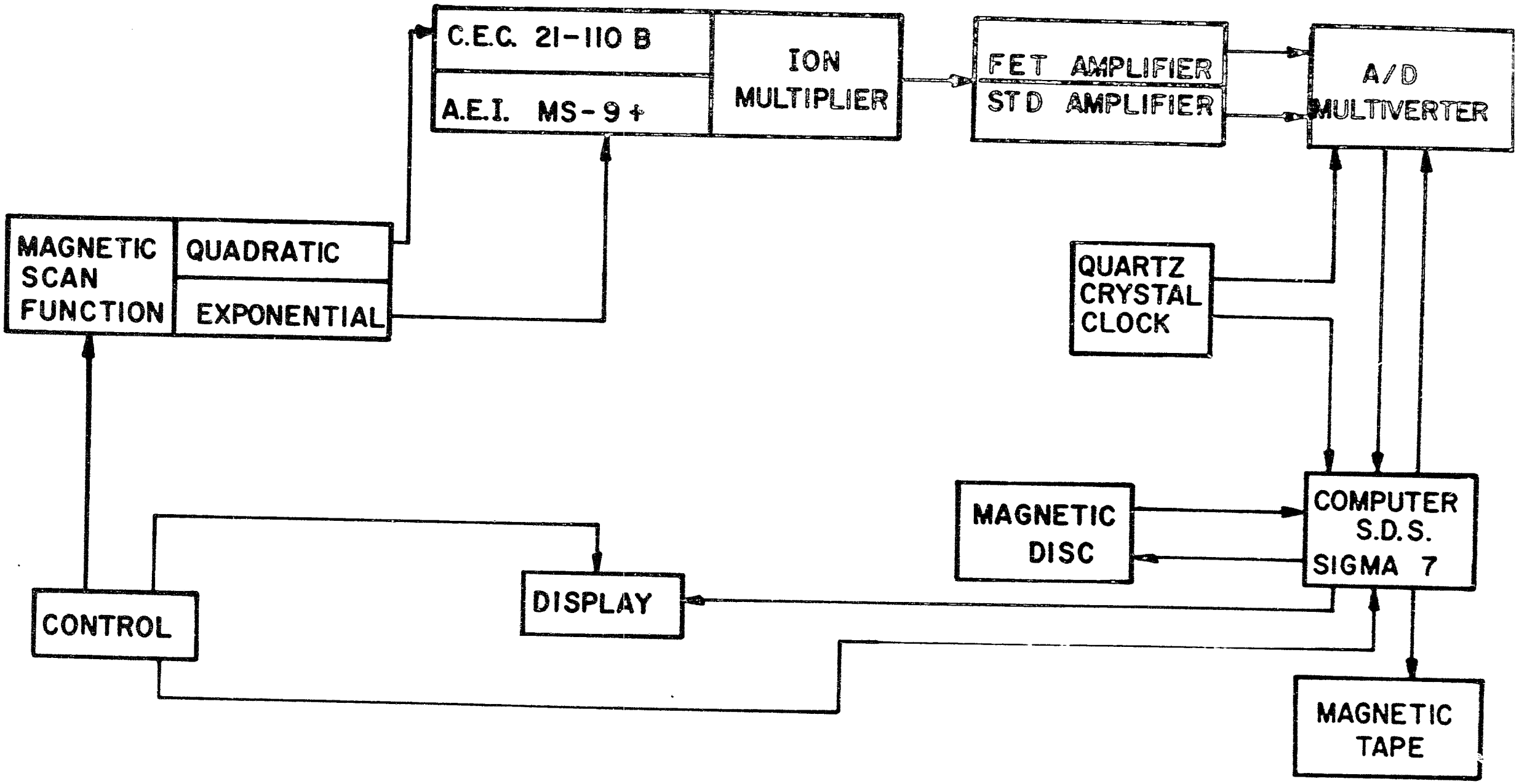
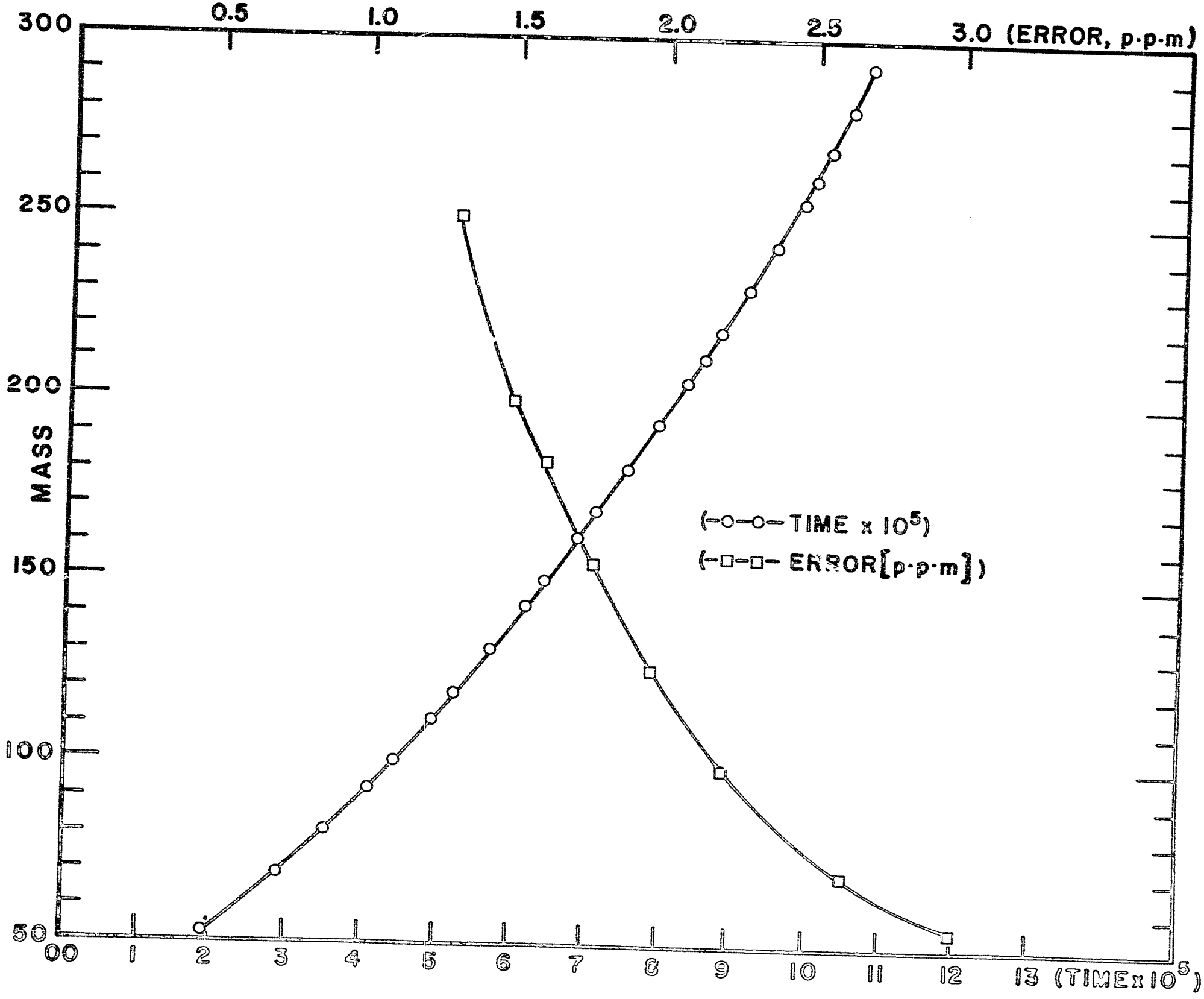
Fig. 1 DATA ACQUISITION HARDWARE

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(-□-□- ERROR[p·p·m])
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2
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6
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9
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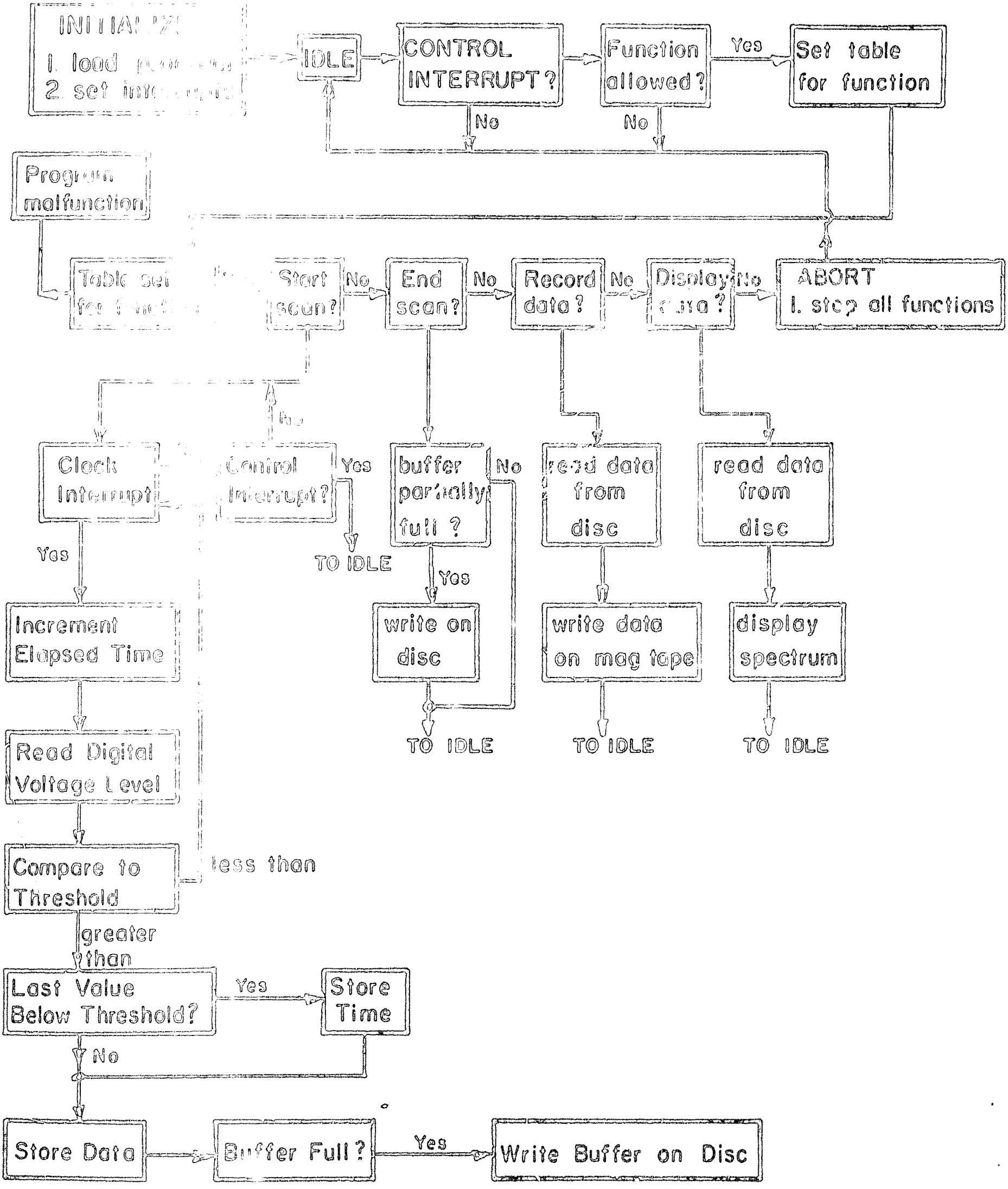
11
12
13 (TIME x 10^5)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Record data?
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No
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Control Interrupt?
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read data from disc
Yes
Increment Elapsed Time
write on disc
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display spectrum
TO IDLE
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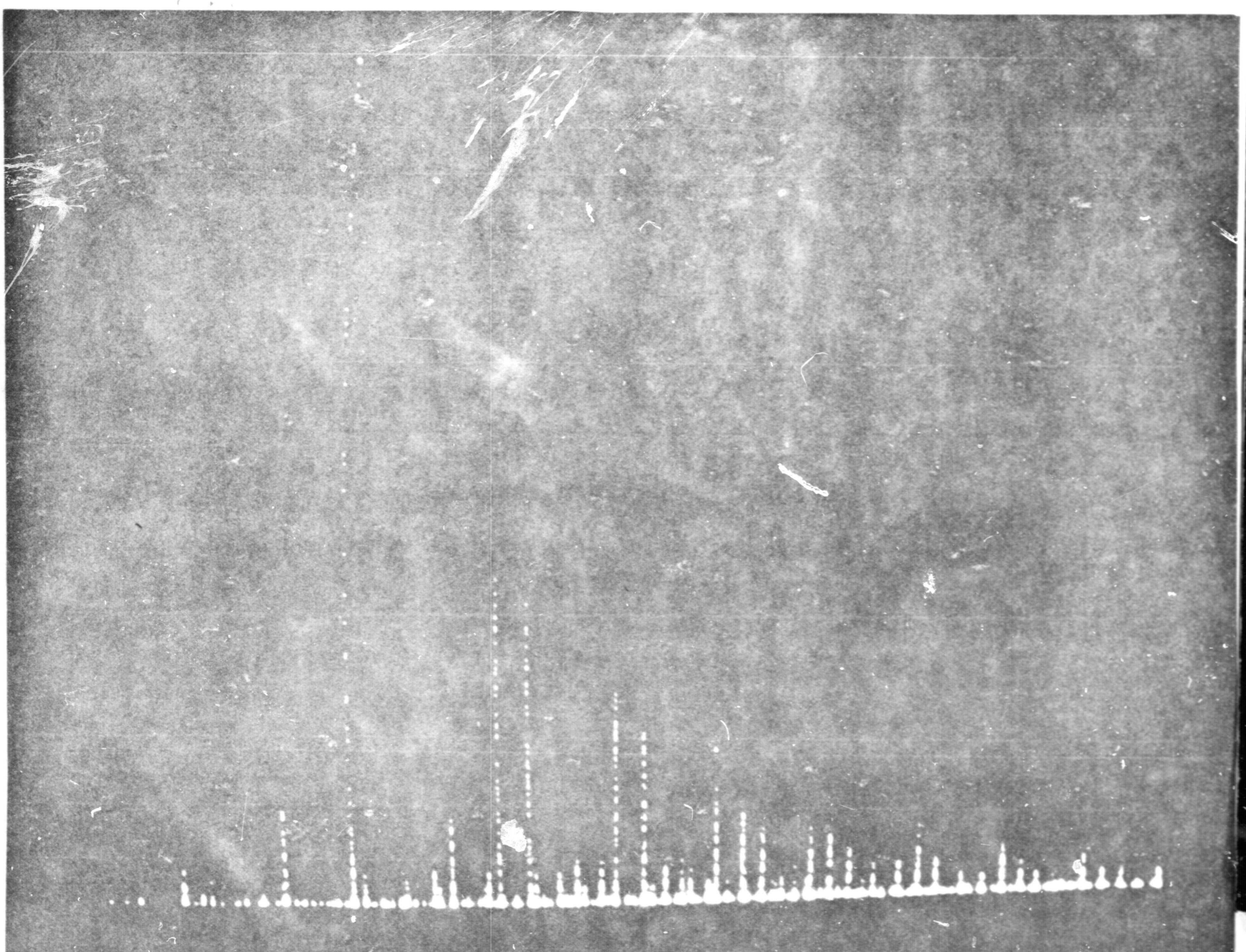
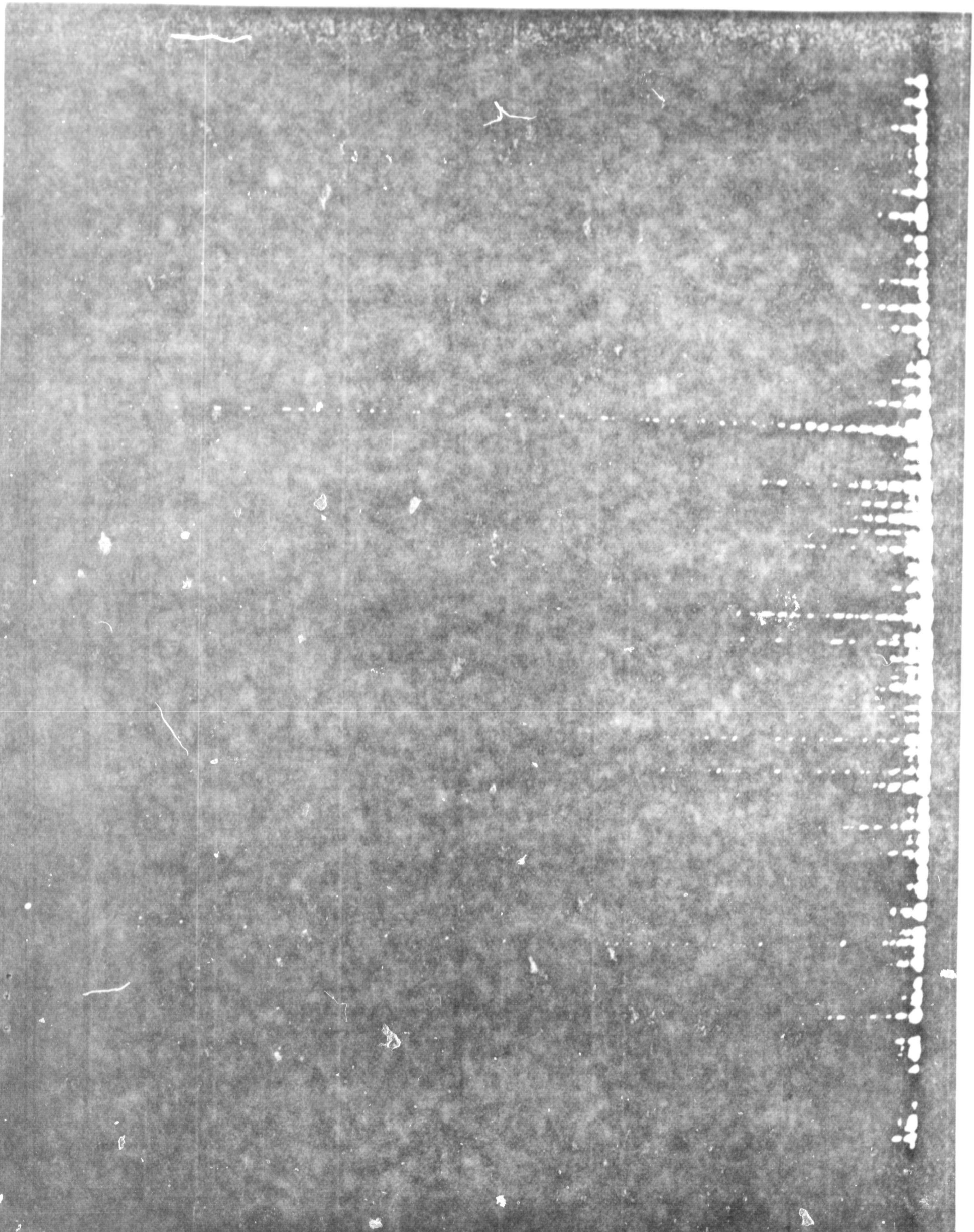
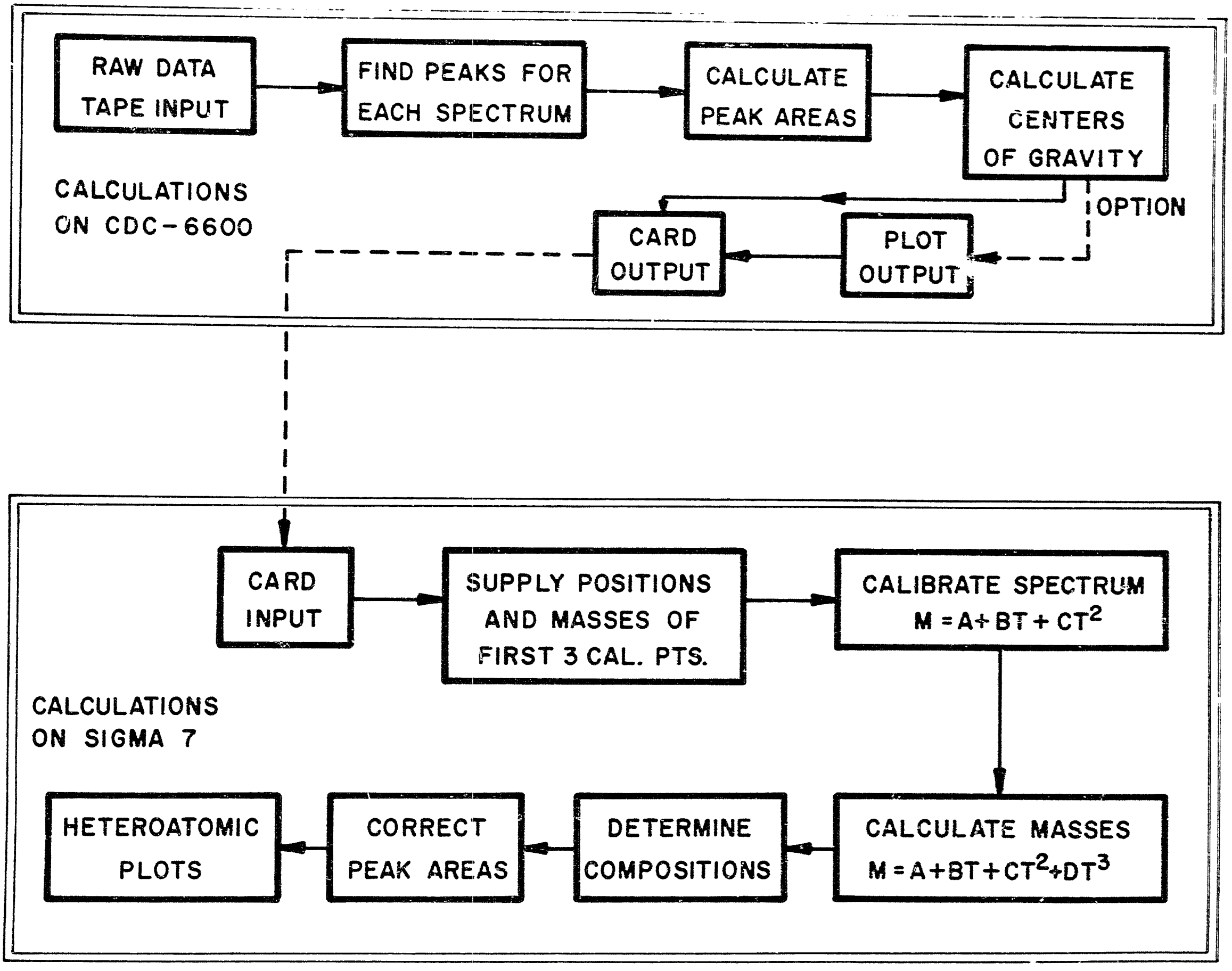
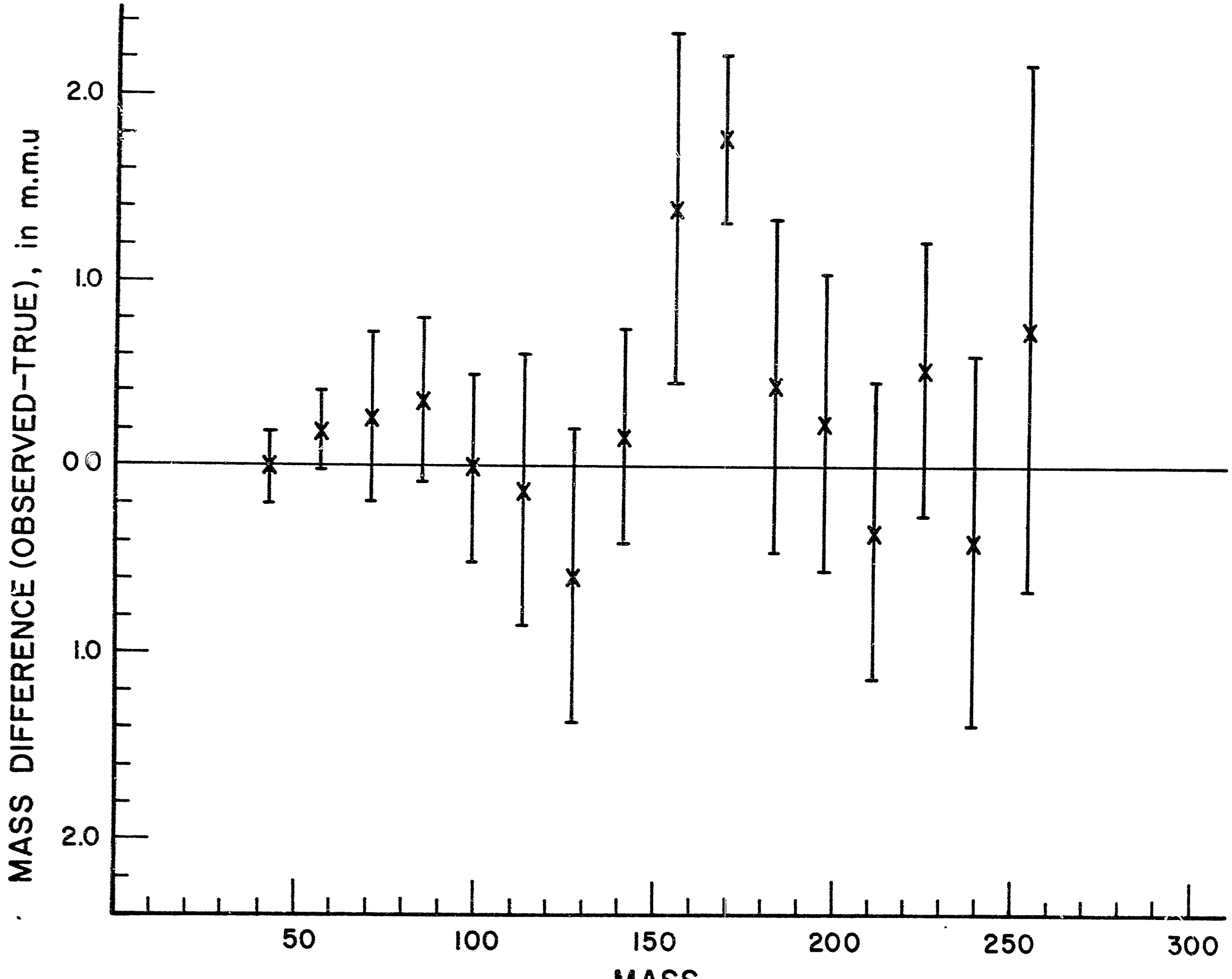
Write Buffer on Disc

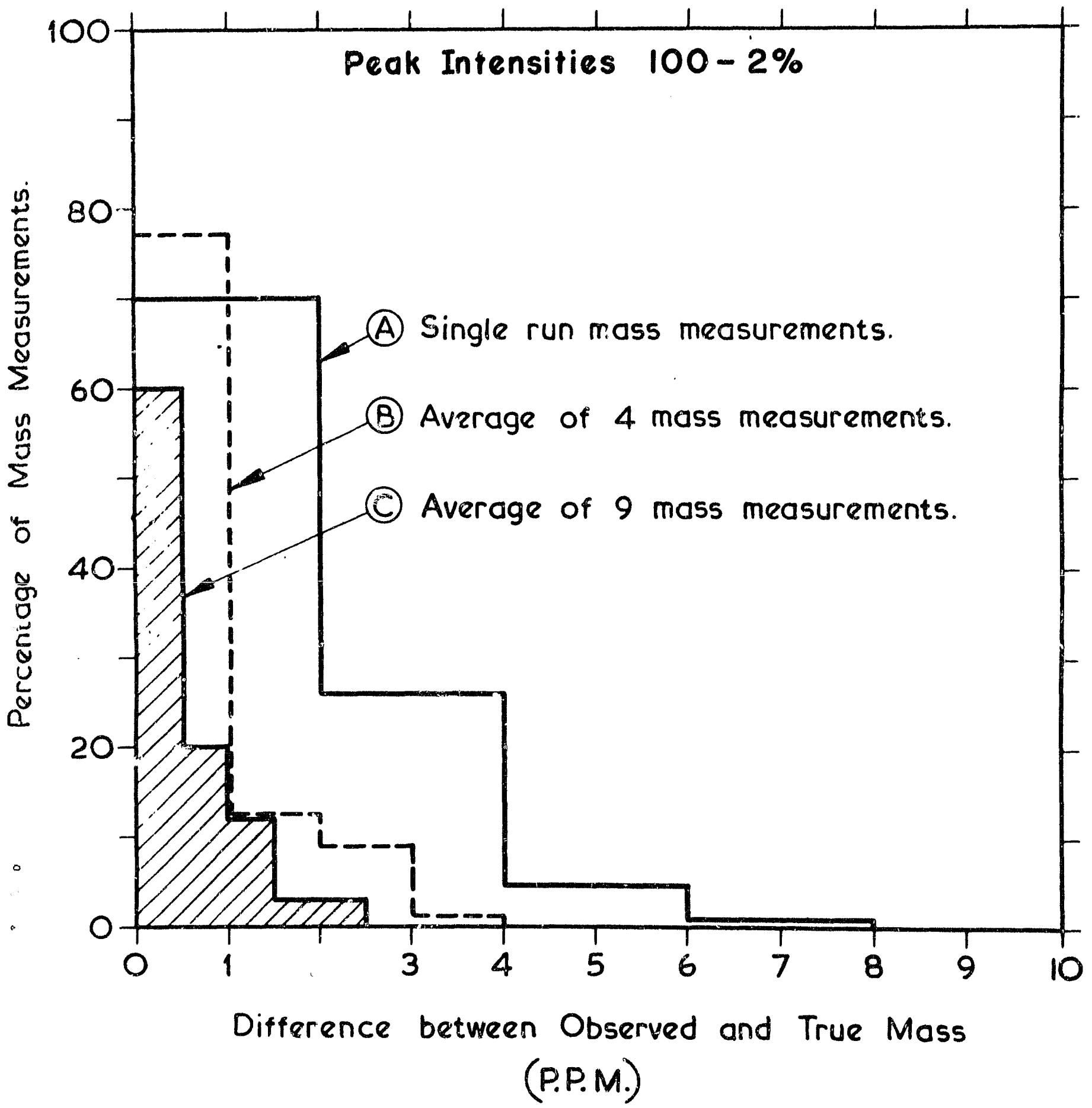


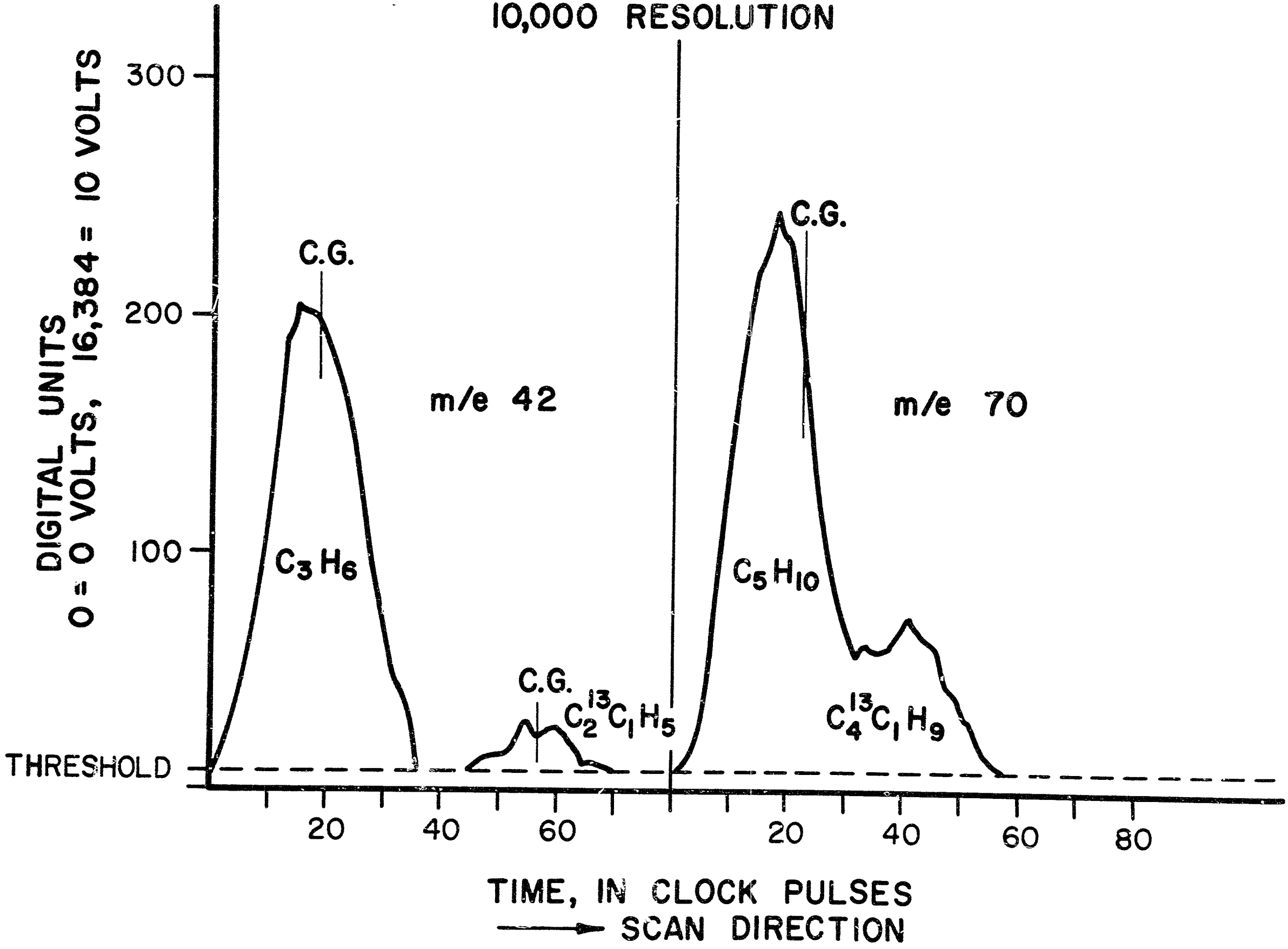


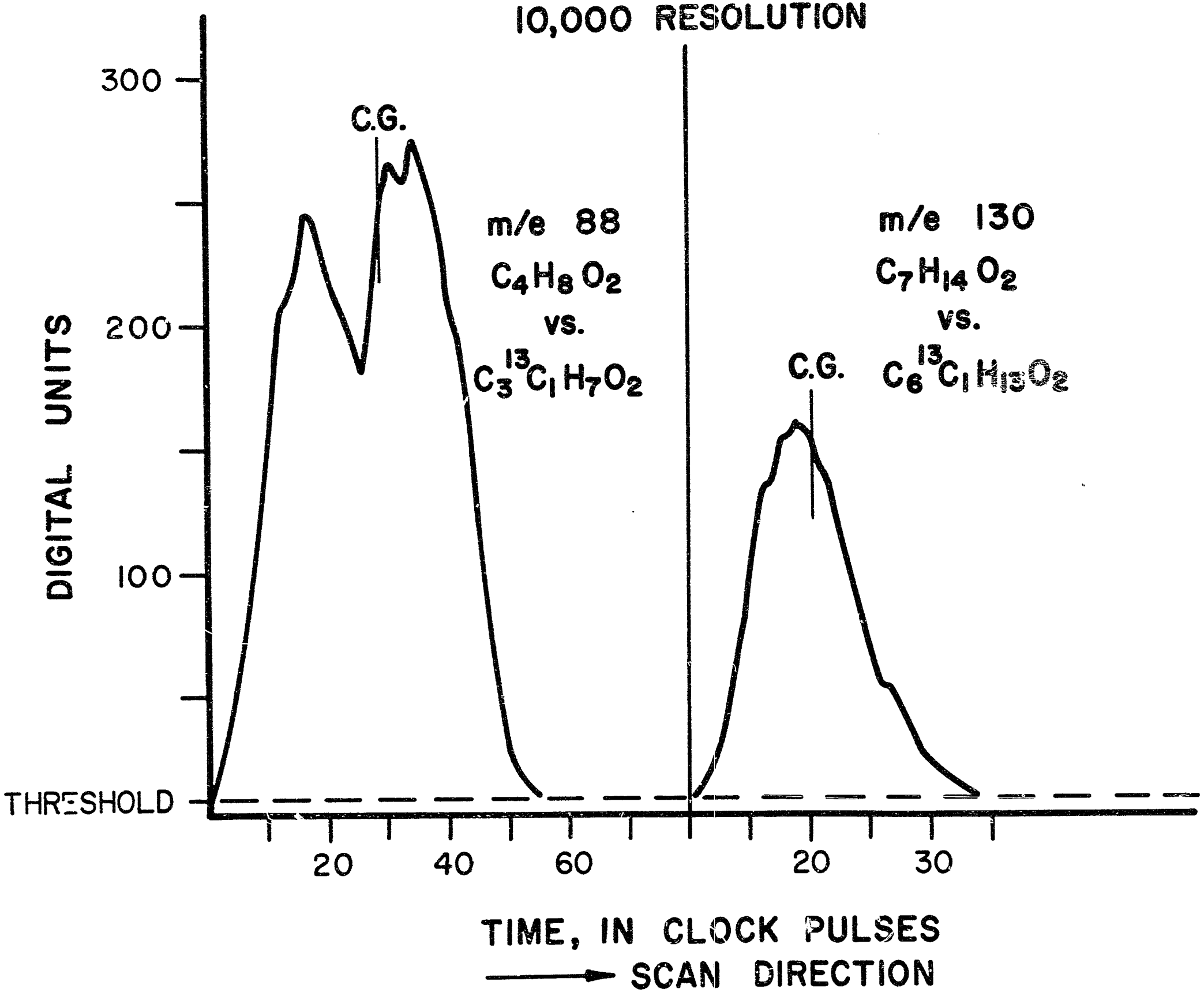

RAW DATA TAPE INPUT
FIND PEAKS FOR EACH SPECTRUM
CALCULATE PEAK AREAS
CALCULATE CENTERS OF GRAVITY
CALCULATIONS ON CDC-6600
OPTION
CARD OUTPUT
PLOT OUTPUT
CARD INPUT
SUPPLY POSITIONS AND MASSES OF FIRST 3 CAL. PTS.
CALIBRATE SPECTRUM M = A + BT + CT2
CALCULATIONS ON SIGMA 7
HETEROATOMIC PLOTS
CORRECT PEAK AREAS
DETERMINE COMPOSITIONS
CALCULATE MASSES M = A + BT + CT2 + DT3

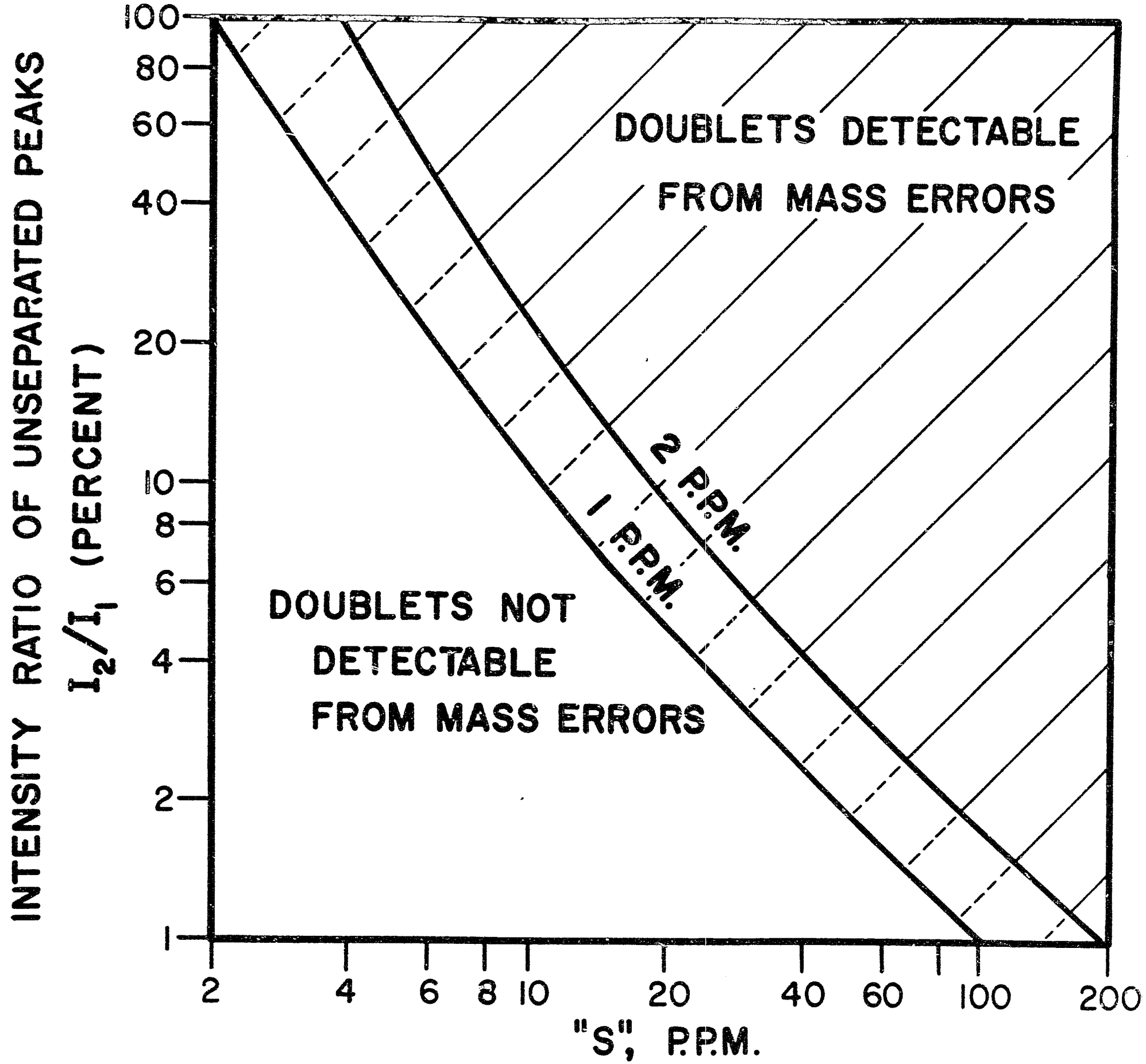

MASS DIFFERENCE (OBSERVED–TRUE), in m.m.u
2.0
1.0
0.0
1.0
2.0
50
100
150
200
250
300
MASS

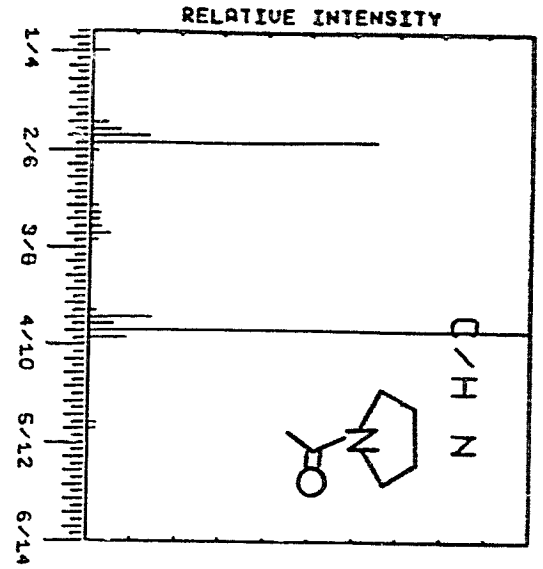
DISTRIBUTION OF MASS MEASUREMENT ERRORS AT RESOLUTION OF 25,000

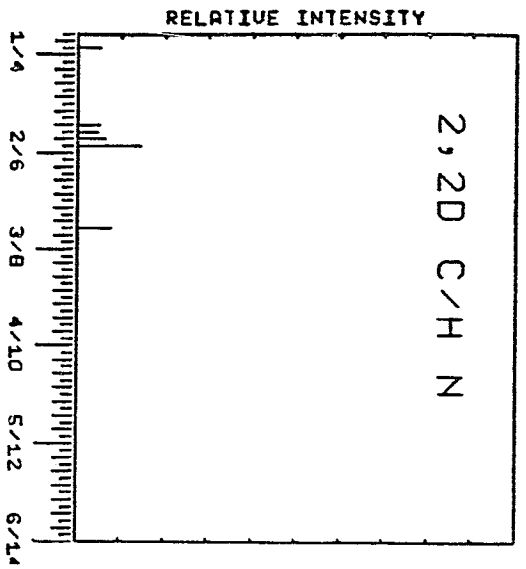


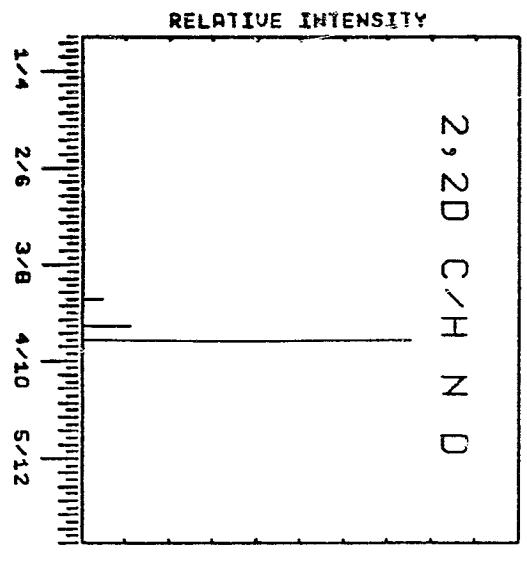

10,000 RESOLUTION
DIGITAL UNITS
0 = 0 VOLTS, 16,384 = 10 VOLTS
300
200
100
THRESHOLD
C.G.
m/e 42
C_3H_6
C.G.
$C_2^{13}C_1H_5$
m/e 70
C.G.
C_5H_{10}
$C_4^{13}C_1H_9$
20
40
60
20
40
60
80
TIME, IN CLOCK PULSES
SCAN DIRECTION

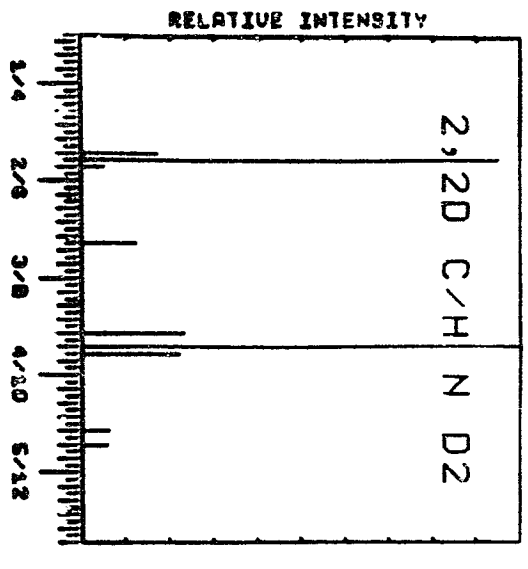

10,000 RESOLUTION
300
200
100
THRESHOLD
DIGITAL UNITS
C.G.
m/e 88
$C_4H_8O_2$
vs.
$C_3{}^{13}C_1H_7O_2$
C.G.
m/e 130
$C_7H_{14}O_2$
vs.
$C_6{}^{13}C_1H_{13}O_2$
20
40
60
20
30
TIME, IN CLOCK PULSES
SCAN DIRECTION

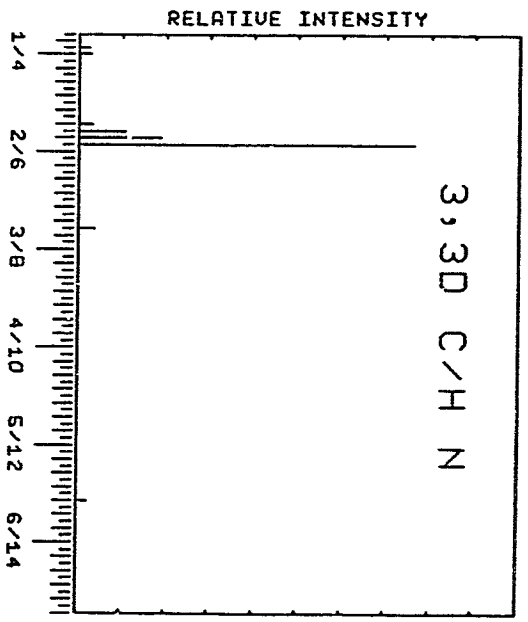

INTENSITY RATIO OF UNSEPARATED PEAKS
I_2/I_1 (PERCENT)
100
80
60
40
20
10
8
6
4
2
1
DOUBLETS DETECTABLE
FROM MASS ERRORS
2 P.P.M.
1 P.P.M.
DOUBLETS NOT
DETECTABLE
FROM MASS ERRORS
2
4
6
8
10
20
40
60
100
200
"S", P.P.M.

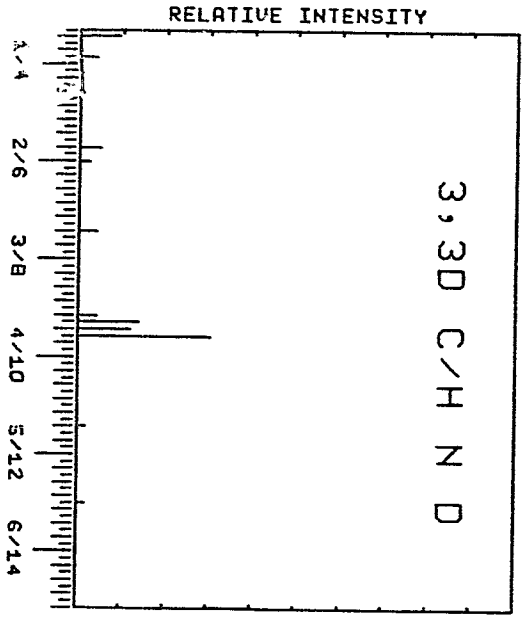

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14
C/H N

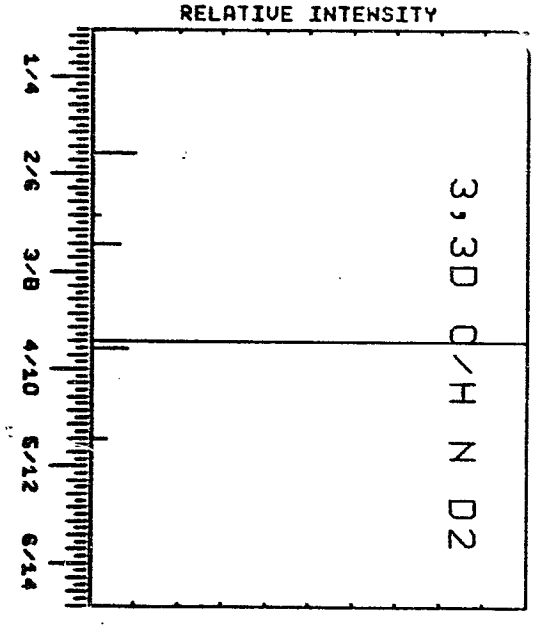

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14
2,2D C/H N

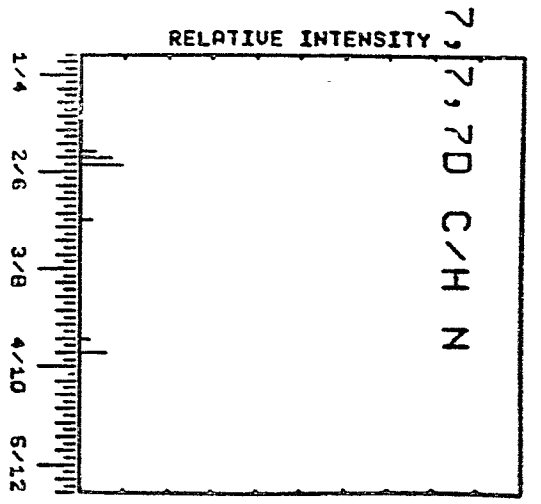

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
2,2D C/H N D

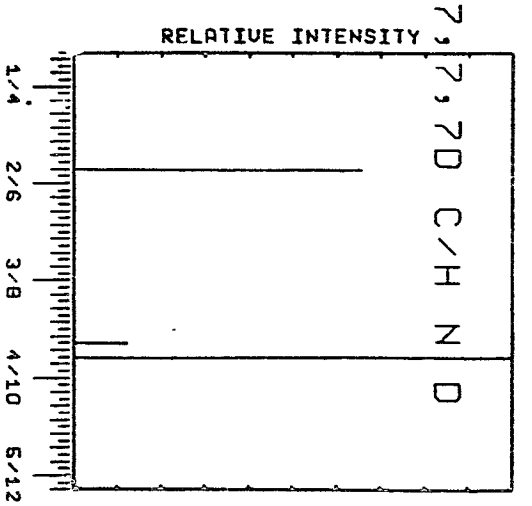

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
2,2D C/H N D2

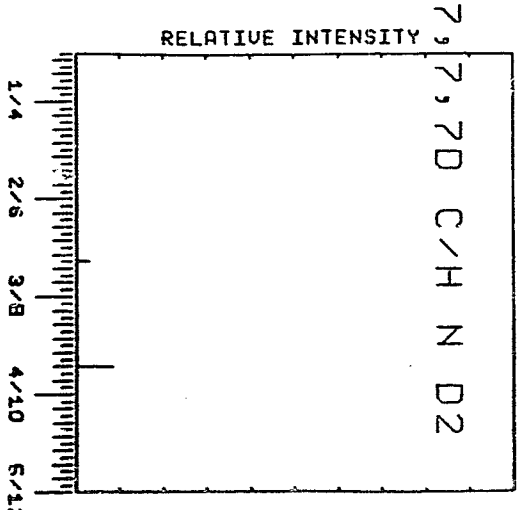

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14
3,3D C/H N

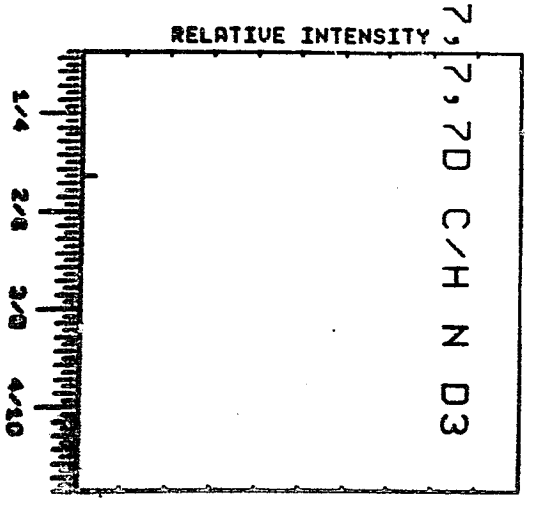

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14
3,3D C/H N D

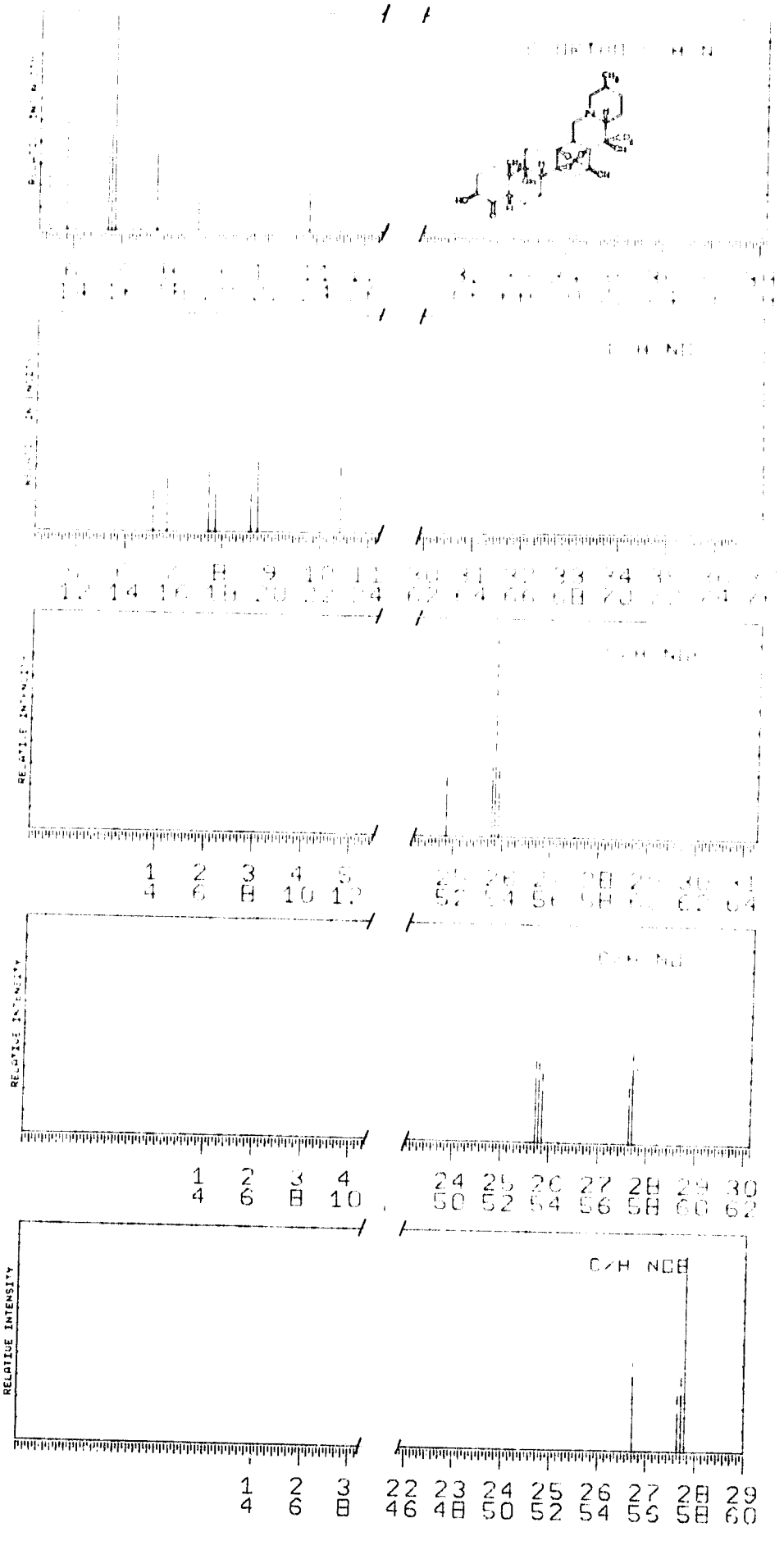

RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
6/14
3,3D C/H N D2


RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
7,7D C/H N


RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
7,7D C/H N D


RELATIVE INTENSITY
1/4
2/6
3/8
4/10
5/12
7,7D C/H N D2


RELATIVE INTENSITY
1/4
2/6
3/8
4/10
7,7D C/H N D3


RELATIVE INTENSITY
C/H NCH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64

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